STUDY OF GROWTH AND BONE MINERAL DENSITY AND FACTORS AFFECTING THEM IN CHILDREN AND ADOLESCENTS WITH THALASSAEMIA MAJOR AND SICKLE CELL DISEASE.

Submitted by:

Ashraf T. M *Soliman MD*
Associate Professor of Paediatrics
College of Medicine
University of Alexandria, Alexandria, Egypt.

To

The University of Glamorgan

In Partial fulfilment of the degree of
Ph.D. in Paediatrics

Supervisor:

Behjat M Ansari FRCPCH
Professor of Paediatrics
University of Glamorgan,
Head Academic: Department of Paediatrics,
East Glamorgam General Hospital
Church Village

1998

FOREWORD

Thalassaemia and sickle cell disease (SCD) are the most widely distributed blood genetic disorders that occur at a high frequency in some populations including the Mediterranean region, parts of the Middle East, South East Asia and the Indian subcontinent. It is estimated that thalassaemia major affects 100,000 new-born every year world-wide. The high incidence of these chronic haemolytic diseases in developing countries poses a high load on the national economy because of the expensive treatment protocols and the considerably high morbidity rates of these patients. Repeated blood transfusion to keep haemoglobin above an acceptable level requires well-equipped blood banks with expensive facilities to screen, store and manipulate blood and blood products. Iron chelation therapy is an essential part of treatment to avoid or delay the deleterious effects of iron overload on different organs including the liver, heart, pancreas and endocrine glands. This requires injecting deferoxamine subcutaneously for 12 hours daily with a special pump. Both deferoxamine and pumps are expensive and therefore not accessible for all patients.

In developing countries, the majority of transfusion-dependent patients with chronic haemolytic anaemia (thalassaemia and SCD) suffer from the consequences of sub-optimal treatment. The mortality rate is still high and usually patients die before the age of 30 years. They also suffer from chronic multi-organ damage including cardiac failure, liver cirrhosis, insulin-dependent diabetes mellitus, growth and pubertal failure and many skeletal abnormalities and fractures. In developed countries the introduction of high transfusion regimes and efficient chelation therapy improved survival rates and prevented cardiac and hepatic damage. However, a majority of thalassaemic patients still have significant growth and pubertal abnormalities, bone disease and multiple endocrine disorders.

In Egypt the incidence of thalassaemia major ranges between 0.1 - 0.2% which gives very high patient load on the medical services. In our University of Alexandria Children's Hospital, Alexandria, Egypt. The Haematology clinic has an average of 150 thalassaemic children registered. The same problem is encountered by me in the Royal Hospital, Muscat, Oman, with high prevalence of SCD and thalassaemia and suboptimal treatment. Because of the restricted economic resources, both hospitals adopt a low transfusion therapy (to keep haemoglobin above 9 g/dl) with IM chelation 3 times per week. With this form of sub-optimal treatment we observed that a large number of our thalassaemic children have severe growth and pubertal failure/delay, beside other hepatic, cardiac and skeletal abnormalities. In fact they constitute 40% of patients attending our Endocrinology clinic. This stimulated me to perform an extensive study to survey growth and pubertal development in theses patients (study-1) and investigate the different factors that might affect their growth and pubertal development (studies 4 through 10) a well as bone mass density (studies 11,12). The frequent involvement of the liver in these patients led us to study some hepatic functions and the prevalence of transfusion-associated hepatitis B surface antigenaemia and hepatitis-C virus antibody seropositivity in relation to their

linear growth (studies 2,3). We studied the nutritional intake of these patients, their intestinal absorption of D-Xylose and 48-h stool fat content in relation to their body mass index, subcutaneous fat thickness and mid-arm circumference (studies 4,5,9).

Their defective linear growth urged us to investigate their growth hormone (GH) secretion (spontaneous nocturnal as well as after provocation) and insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 (IGFBP3) concentrations. Our findings demonstrated high prevalence of defective GH secretion in these children that necessitated imaging of their hypothalamic pituitary area. Imaging studies revealed original data about structural abnormalities in the anterior pituitary gland, different degrees of pituitary atrophy and empty sella and infiltration the gland as well as the mid-brain by haemosidrin in thalassaemic children, the mechanism of these findings was explained (studies 4-6, 10). Because of their slow growth, the presence of abnormal GH/IGF-I/BP3 axis, and structural abnormalities of the pituitary gland, the next step dealt with the response of IGF-I to exogenous GH and the clinical response of their linear growth to GH therapy for a year or more (studies 4,9).

Based on the fact that these patients have high prevalence of bone pains and osteoporosis during late childhood and have high risk of spontaneous fracture thereafter, we measured their bone mass density to investigate the relation between the former and the degree of iron load, growth parameters, and different anabolic hormone concentrations in these patients (studies 11,12).

The overview contains the details of all the 12 studies, summary and recommendations. For tables and figures please refer to the original papers (1-12) included in the APPENDIX.

CONTENTS

INTRODUCTION

The thalassaemias and sickling disorders are genetic disorders of haemoglobin synthesis that constitute the most widely distributed blood genetic disorders and occur at a high frequency in some populations including the Mediterranean region, South-East Asia, the Indian subcontinent and parts of the Middle East (1-3).

I) Beta- thalassaemias:

The thalassaemia syndromes are diverse group of genetic disorders of haemoglobin (Hb) synthesis. The overall effect is characterised by reduced rate of synthesis of one or more globin chains of Hb giving rise to an imbalance in the alpha-globin to non-alpha-globin chain ratio (1). The excess chains frequently associate to form unstable tetramer of similar chains (for example gamma 4, beta 4) and precipitate in the red cells thereby producing red cell haemolysis. Due to the decreased synthesis of Hb, the red cells typically appear pale (hypochromic) and are small (microcytic) in size. The degree of hypochromic-microcytic anaemia varies and ranges from a mild state to a severe, transfusion-dependent syndrome. These disorders constitute one of the most widely distributed blood genetic disorders and occur at a high frequency in some populations, particularly those with past or present history of malaria endemicity. These include the Mediterranean region, South-East Asia, the Indian subcontinent, part of the Middle East and in individuals of African origin (2).

The thalassaemias are classified into groups depending on the type of globin chain, which is deficient or not synthesised. The major types of thalassaemias α, β, δ, $\delta\beta$ or $\gamma\delta\beta$ thalassaemias, there is decreased production of α, β, δ, $\delta+\beta$ or $\gamma+\delta+\beta$ globin chains, respectively. The most frequently encountered thalassaemias are the α- and β-thalassaemias. Other occur at low frequency in some populations. Within each type of the thalassaemia, further classification is based on: the genotypic abnormality and phenotypic abnormality (3).

The β-thalassaemias result from point mutations or deletion of part or whole of the β-globin gene cluster. Over one hundred different mutations causing β-thalassaemia have now been identified. Unlike the α-thalassaemias, the majority of the β-thalassaemias result from point mutations, in which the β-globin gene and its intervening or flanking sequences are intact. However, the rate of synthesis of mRNA, its processing or stability

may be reduced, resulting in decreased β-globin synthesis (4,5). The non-deletional β-thalassaemias result from over 95 point mutations, affecting different parts of the β-globin gene or its regulatory regions. The resulting pattern may be β+ type i.e. when there is some β-globin chain synthesis, or βo-type i.e. where there is no β-globin chain synthesis. Every population that has so far been investigated has six to eight common mutations more or less specific to that population, while other mutations occur at a very low frequency. Deletion of part or whole of the β-globin gene cluster have been reported in different populations. Depending on the extent of deletion the thalassaemia may be βo, (δβ)o, (γδβ)o, or (εγδβ)o thalassaemia. The (δβ)o thalassaemias have a functional γ globin gene and often result in hereditary persistence of foetal haemoglobin (HPFH) (6,7). Prenatal diagnosis is possible , usually by PCR or Southern blotting, carried out on foetal DNA obtained by chorio-villous sampling between 11 and 15 weeks gestation. Later diagnosis is possible via amniocentesis or foetal blood sampling.

Phenotypically the β-thalassaemias present as β-thalassaemia minor, β-thalassaemia intermedia and β-thalassaemia major (8,9):

1. The β-thalassaemia minor (or trait): in general the minor type is clinically asymptomatic and the red cells are microcytic and hypochromic. But erythrocytosis and poikilocytosis are observed. The Hb A2 level is generally elevated but may be normal. The β-thalassaemia minor occurs in individuals heterozygous to β+ or βo-mutations. The former are generally not anaemic, but the latter have a mild degree of anaemia.
2. β-thalassaemia intermedia: the condition is associated with splenomegaly and haemolytic anaemia of hypochromic and microcytic type and some patients may require blood transfusion. There is a decreased production of Hb A and in majority of the cases an elevation of Hb F. Some of the clinical abnormalities listed with β-thalassaemia major are also encountered in these patients, but the condition is generally milder.
3. β-thalassaemia major: this is the most serious consequences of β-thalassaemia mutations and is associated with complete absence, or very low production, of Hb A. Clinically several abnormalities are apparent in these patients. In general, there is associated hepatosplenomegaly, and marked bone changes. The patients require blood transfusion due to severe chronic haemolytic anaemia. Blood film reveals α-chain inclusion bodies in hypochromic and microcytic red cells. The major clinical findings of thalassaemia major include: pallor and jaundice, splenomegaly,

hepatomegaly, transfusion-dependence, skeletal and dental changes, growth retardation, gallstones, skin pigmentation, cardiac complications, and susceptibility to infections.

Homozygous βo thalassaemia usually becomes symptomatic as severe, progressive haemolytic anaemia during the 2nd 6 months of life. Regular blood transfusions are necessary in these patients to prevent profound weakness and cardiac decompensation caused by the anaemia. In untreated cases or in those receiving infrequent transfusions at times of severe anaemia, hypertrophy of erythropoeietic tissue occurs in medullary and extramedullary locations. The bones become thin and pathologic fractures may occur. Massive expansion of the marrow of the face and skull produce characteristic facies. Pallor, haemosiderosis and jaundice combine to produce a greenish brown complexion. The spleen, liver and lymph nodes are enlarged by extramedullary-haematopoiesis and haemosiderosis. In older patients the spleen may become so enlarged that it causes mechanical discomfort and secondary hypersplenism . Growth is impaired in older children; puberty is delayed or absent because of secondary endocrine abnormalities. Diabetes mellitus resulting from pancreatic siderosis may occur. Cardiac complications, including intractable arrhythmias and chronic congestive failure caused by myocardial siderosis, are common terminal events. With modern regimens of comprehensive care including hypertransfusion and supertransfusion protocols and proper iron chelation therapy , many of these complications can be prevented and others ameliorated and delayed in their onset (9).

The β thalassaemias occur in populations from the Mediterranean , Africa, the Middle East, India, Pakistan, and China. The geographic distribution of thalassaemia bears a striking similarity to that for malaria (8). In Egypt, the prevalence of β thalassaemia minor ranges between 4.2 and 7 per cent (10,11). This value is similar to that reported from North and West Africa (7-8 per cent) (12). The prevalence of β thalassaemia major in Egypt ranges between 0.1 to 0.2 per cent with a gene frequency between 0.01 per cent and 0.04 per cent in different regions (10,11,13).

Iron overload:

Generalised iron loading of the organs has been a recognised complication of thalassaemia . The excess iron is derived both from transfusion and from intestinal absorption; in adequately transfused children the former mechanism predominates. The degree of iron overload derived from blood transfusion obviously depends on the

type of transfusion regime. Each 500 ml of blood delivers about 200 mg of iron to the tissues that can not be excreted by physiologic means. Myocardial siderosis is a significant contributing factor in the early death of these patients. Whereas siderosis of the liver, pancreas, renal and endocrine glands markedly contribute to the morbidity of these patients (1,3,8,14). Haemosiderosis can be decreased or even prevented with the parenteral administration of the iron-chelating drug, deferoxamine. It is administered subcutaneously over 12-hr period using a small portable pump (during sleep) 5 or 6 nights/week to keep serum ferritin levels below 1000 ng/ml, which is well below the toxic range. The proper use deferoxamine has been shown to delay the development of iron induced damage of cardiac and liver tissue, resulting in improved survival. The ability of deferoxamine to prevent endocrine damage is less clear (15, 16).

II) Sickling Disorders:

Include the carrier trait for HbS (AS), the homozygous state or sickle cell disease (SS), or the co-inheritance of the sickle cell gene and other haemoglobin variants, for example: HbC, giving sickle cell haemoglobin C disease, and sickle cell-βo or sickle cell-β+ thalassaemia. There are marked haematological and clinical differences between these genotypes (17,18).

Sickle haemoglobin (HbS) differs from normal adult haemoglobin by a substitution of glutamic acid at the 6^{th} position of its β chains by valine. In the oxygenated state Hb S functions normally. When this haemoglobin is deoxygenated, an interaction between the β6 valine and a complementary region on the β chains of an adjacent molecule results in the formation of highly ordered molecular polymers; these elongate to form filamentous structures, which aggregate into rigid , crystal-like rods. This process of molecular polymerisation is responsible for the spiny, brittle character of sickle erythrocytes under conditions of decreased oxygenation (8).
Certain other abnormal haemoglobins, notably Hb C , Hb D Los Angeles, and Hb O Arab, participate in the molecular polymerisation of deoxy Hb S. Hb A does so to a smaller degree, but foetal haemoglobin (Hb F) does not. Hb S is readily identified, as early as the first day of life, by electrophoresis. A confirmatory solubility test excludes other abnormal haemoglobins with similar electrophoretic mobility. These disorders can also be determined antenatally using amniocyte or chorionic villous DNA by methods that identify the specific βsS nucleotide substitution (19,20)

Polymerisation of Hb S molecules within the red cell membrane so that Hb S containing cells are less able to negotiate capillary beds leading to premature red cell destruction (haemolysis) and obstruction of blood flow (vaso-occlusion) attribute to most manifestations of the disease. Haemolytic anaemia usually gradually develops over the first 2-4 months, paralleling replacement of much of the foetal haemoglobin by Hb S. The consequences of chronic haemolysis include anaemia, jaundice, pigmented gallstones, increased folic acid requirements with a tendency to folate deficient megaloblastic change, the aplastic crisis, and retarded growth and sexual development. The consequences of vaso-occlusion are determined by the site affected and include stroke, ocular complications, splenic damage with subsequent tendency to acute splenic sequestration and pneumococcal septicaemia, acute chest syndrome, leg ulceration, priapism, bone and bone marrow necrosis and possibly renal failure. Both erythopoietic expansion secondary to haemolysis and vaso-occlusion may contribute to the avascular necrosis of bone resulting in dactylitis (hand- foot syndrome) , painful crisis, avascular necrosis of the femoral head and other bone damage. The painful crisis and leg ulceration are major contributors to morbidity but rarely cause death. The major causes of death include acute splenic sequestration , pneumococcal septicaemia acute chest syndrome and stroke (21-25). Cardiomegaly is invariably present in older children, often caused partly by sickle-related cardiomyopathy. Increased iron absorption contributes to parenchymal damage of the liver, pancreas and heart. By mid-childhood most patients are underweight, and puberty is frequently delayed (8).

The sickle gene is found in high frequency in those living in regions in which Plasmodium falciparum malaria has been endemic, reaching its highest incidence in equatorial Africa but occurring also in the Mediterranean area, Southern Turkey, the Middle East, Saudi Arabia, and much of Central India (8,18). In Egypt the prevalence of sickle cell trait differs considerably among the different regions of the country. In Alexandria (on the Mediterranean sea), the prevalence is 1%, similar to those found in Syria and Lebanon. (10,26) Whereas in certain areas of Egypt, especially Siwa, El-Karga and El-Dakhla Oasis, the prevalence ranges between 9% 22.2% . For centuries these oasis have been an important cross road for the Caravans linkage of Egypt and Sudan (27,28).

Normal growth and pubertal development and their endocrine control:

A description of the endocrine control of growth requires a model which embodies the different phases of growth which have important biological connotations and significant changes that occur at crucial points in a child's development. Important clinical clues to the aetiology of a growth problem in an individual child can easily be lost from the rather static approach suggested by descriptive models.

The human growth curve comprises at least three distinct functions and our understanding of the hormonal regulation of growth concurs with this. Analytic descriptions of the curve include three components of the model and the hormones most likely to be involved in the expression of each component (29):

1. **The infancy component: is the rapid, but rapidly decelerating, growth of the first 2-3 years of life described by an exponential function representing factors important in foetal growth. It appears to be largely nutritionally determined. Long-term effects on growth are seen following over or under-nutrition at this stage of life. The endocrine control of growth in utero is quite different from that after birth. Major growth promoting hormones such as growth hormone (GH) thyroxin, and sex steroids have almost no influence on foetal growth (30). In contrast, insulin is essential for foetal growth by acting as mitogen on embryonic tissues (31), and via stimulation of tissue insulin-like growth factor-I (IGF-I) release (32). IGF-I controls organ growth and functional differentiation during foetal growth (33,34). These facts explain macrosomia in infants of diabetic mothers and those with nesidioblastosis(35,36). However, after birth dramatic changes occur with the appearance of a quite different physiology. Circulating insulin concentration declines, and IGF-I secretion gradually becomes GH dependent and less dependent on insulin (31,37). These changes might explain in part the deceleration of growth in the infancy component of the growth curve.**

2. **The childhood component: is represented by a second degree polynominal regression, the earliest onset of which could be recognised at 6 months of age. Therefore, until the age of 3 years, growth is a combination of the infancy and childhood components acting additively. Such a statement is supported by the study of children with GH deficiency**

(particularly where there is gene deletion) who show a growth pattern compatible with the infancy component. The childhood component is therefore largely determined by GH secretion (GH/IGF-I axis). During childhood, stature is determined by the size that an infant has reached by the end of the first year of life, which is determined partly by the genetic circumstances and influenced greatly by nutrition, and the rate at which the child grows thenceforward. The rate is related in an asymptotic fashion to the amount of GH secreted. (38) No relationship was found with cessation of breast feeding, season of the year, social group or mid-parental height (29).

3. **<u>The pubertal component</u>: when growth is described by a logistic function dependent endocrinologically on a combination of GH and sex steroids. This is superimposed on the decelerating childhood component which explains the fact that the magnitude of the adolescent growth spurt is inversely related to the age at peak height velocity. The contribution of the pubertal component is independent of time: what constitute the difference is the point at which it is superimposed on the decelerating childhood component. The puberty component depends on sex steroids which have both a direct anabolic action and a modulating effect on GH secretion (29).**

It is evident that any endocrine account on linear growth must explain the three phases of that process and must view hormone action as an integrative effect rather than the contribution of distinct entities.

A) The hypothalamic-pituitary-somatotropic growth axis during childhood:

The principal hormone influencing mid-childhood growth is GH, the secretion of which is regulated by two hypothalamic peptides, growth hormone releasing hormone (GHRH) and somatostatin. GHRH controls synthesis of GH while somatostatin modulates its pulsatile secretion (29,30,39-43).

The human GH (hGH) gene is present on chromosome 17 at band q2.3, along with the two genes for human somatomammotrophin. Both hormones have 191 amino acids and there is 85 per cent homology between the amino acid residues. The currently used hGH gene nomenclature refers to the hGH1 and hGH2. The hGH1 gene and its messenger RNA transcript have five exons separated by four interons. Transcription of this gene leads to the synthesis of 22 kb hGH which accounts for 90 per cent of the hGH synthesis. The excision of the second interon of the hGH1 gene leads to an alternative splicing site resulting in deletion of the message for amino acid residues 32-46 leading to a smaller molecule termed the 20 kb hGH variant which forms 10 per cent of the GH synthesised (30,39-43).

Growth hormone becomes the predominant modulator of growth velocity towards the end of the first year of extrauterine life. GH is secreted in a pulsatile manner from pituitary somatotroph cell. The majority of GH is secreted during night. In children and young adults, a peak of GH (upto 40 ng/ml) occurs about 1 hour after the onset of deep sleep; other and often smaller peaks can be observed later during sleep. During the day, GH secretion may be related to exercise, stress, or nutrient intake. Such a pulsatile pattern is important for the numerous metabolic actions of GH resulting in normal growth. Normal growth is also dependent on sequential crucial activation events occurring in somatotrophs during development. Pulsatile GH secretion results from interlacing neuroendocrine pathways (39,43-45)

The hypothalamus produces two neuro-hormones that continually influence the overall secretory rate of GH. GHRH, produced in the arcuate nucleus of the medio-basal hypothalamus, stimulates GH release at least in part through the adenylcyclase system as GH release is preceded by an increase in cyclic AMP. In primary culture of cells, treatment with GHRH is associated with an increase in cytosolic GH messenger RNA, a

result similar to that seen after treatment with cyclic adenosine nucleotide cAMP. Somatostatin inhibits GH secretion by inhibiting the production of cAMP . Pulses of GH secretion occur at times of maximal GHRH and minimal somatostatin secretion. Tonic somatostatin secretion is important for maintaining low GH basal levels. Receptors for GHRH and somatostatin are present on somatotropic cells of the anterior pituitary. It is likely that somatostatin and its withdrawal sets the timing of each GH pulse while GHRH determines the magnitude of the subsequent GH peak. Superimposed on this is regulation of somatotroph responsivity by IGF-I (39,43-47)

In addition to the two hypothalamic hormones, many neurotransmitters and neuropeptides are involved in the neural control of GH secretion with both stimulatory and inhibitory effects on GH release. These effects are exerted mainly via GHRH and/or somatostatin rather than directly at the pituitary level. Amid these compounds, neurotransmitters such as noradrenaline/adrenaline, acetylcholine and neuropeptides such as opioid peptides and galanin seem to play an important role. Adrenergic control of GH secretion includes: α-adrenergic, which stimulates, and β- adrenergic, which decreases the secretion. Secretion of GH is stimulated by α-adrenergic agents such as norepinephrine, clonidine and L-dopa, whereas α-adrenergic blockers inhibit the secretion. Opiates and morphine increase GH secretion. Pyridostigmine increases basal GH levels and potentiates GHRH-induced GH secretion. Sertonin is probably involved in the nocturnal secretion of GH (39,45).

GH itself acts on the hypothalamus to affect its own secretion. IGF-I is able to inhibit GH secretion at the hypothalamus level (by stimulating somatostatin secretion) and directly at the pituitary level. Several metabolic and nutritional factors regulate GH secretion. Hypoglycaemia causes GH secretion by still unclear mechanism, probably independent on GHRH. Hyperglycaemia can suppress exercise- or arginine-stimulated GH secretion. Fasting and chronic malnutrition are associated with elevated GH levels. A high-protein diet and some amino acids (arginine) stimulate GH secretion. Increased circulating free fatty acids tend to reduce the GH response to a variety of stimuli (39,48-53)

Circulating hormones can modify GH synthesis and secretion. Thyroid hormones affect the regulation of transcription rate of GH gene. In patients with hypothyroidism, the GH responses to stimuli are decreased; treatment with thyroxin normalises GH secretion. Oestradiol and testosterone increase both basal GH levels and GH release

after arginine infusion or insulin-induced hypoglycaemia. In vitro, glucocorticoids have potent stimulatory effects on GH release, whereas in vivo long -term glucocorticoid administration can suppress GH response to many pharmacological and physiological stimuli. However, short -term administration of dexamethasone can result in stimulation of GH release (39,54-61). Post-transcription mechanisms have been implicated in the positive regulation of human GH by glucocorticoids (62,63).

Specific binding sites for GH have been found in different human tissues; their concentration is high in liver membranes (64). The GH receptor molecule is a polypeptide chain of 620 amino acids with a single transmembrane domain. The extracellular domain of 246 amino acids is heavily glycosylated. The GH receptor gene is on chromosome 5 (5p13.1) and contains nine exons (65). The first demonstrations of GH receptor gene defects have been reported in Laron dwarfs (66). Abnormal regulation of GH gene has been found in several situations including fasting, renal insufficiency and diabetes (39) In the multihormonal control of GH receptors, GH itself and also insulin exert a major role, of up-regulation of GH receptors in most cases (39,64). The high-affinity growth hormone-binding protein (GHBP) in serum is derived from the extracellular domain of the GH receptor and may reflect GH receptor number (65,67) GHBP may confine GH to the intravascular compartment to protect it from degradation and to prolong its biological half-life (68). GHBP is reported to be low in patients with GH deficiency, many patients with idiopathic short stature (69,70) and absent in patients with Laron dwarfism (71). GHBP concentrations positively correlate with height in growing children (72) and with pooled GH values in growing females (73) yet they were found to correlate negatively with 24-hour GH concentrations in boys at mid-puberty (72).This suggests that a higher level of GHBP would decrease the neuroendocrine secretory tone of GH and vice versa, implying that the interaction of GH with GHBP may be more important in modulating the biological effect of GH than the absolute levels alone.

GH acts on many tissues, either directly or through insulin-like growth factor I (IGF-1). Its effects on protein synthesis, carbohydrate and lipid metabolism, and cell differentiation are direct. Alternatively, IGF-I mediates the growth promoting effects. GH exerts its anabolic effects through stimulation of metabolism of nucleic acids and proteins in the liver, the adipose tissue and muscles. GH affects the synthesis of most types of RNA, and it has been shown to increase the transport of α-amino isobutyric acid in the rat diaphragm (39).

Insulin-like growth factors (Somatomedins):

In human serum the IGFs are associated with binding proteins in complexes of 150 and 40 kd. The majority of IGFs are present in the 150-kd complex. After acidification the IGFs are set free and appear as peptides of 7.5 kd. Both for IGF-I and IGF-II the structure is very similar to that of proinsulin. Sequence analysis of cDNA clones encoding the IGFs have confirmed the structure as determined by protein analysis. In addition it was shown that the IGFs are synthesised as preprohormones, which need processing to generate mature IGFs (74,75). This takes place mainly within the tissues. The IGF-I gene is localised on the long arm of human chromosome 12. It is a large chain containing at least 5 exons, four of which contain coding information consisting of a signal peptide, the mature IGF-I (70 amino acids) and a carboxyl-terminal tail (the precursor domain) (74,76,77).

The liver is the major source of somatomedins appearing in blood. Its low level of expression in foetal life switches toward higher expression postnatally. In liver and many other tissues the GH-dependence of IGF-I production has been demonstrated. Peak levels of IGF-I mRNA in all tissues' studies are reached within 3 to 9 hours after a single GH injection, both in GH-deficient and normal animals (78). All tissues respond but its magnitude varies as well as the type of messenger RNA produced. GH is the major regulator of IGF-I production. A 10-fold elevation above normal is observed in patients with acromegaly, whereas low levels are observed in GH-deficient patients (79-80). The responsiveness is also dependent on the presence of mature GH receptors (81). Until puberty a gradual increase of serum IGF-I occurs (82). At puberty, a rise in plasma level occurs (83). Testosterone appears not to have a direct effect on IGF-I levels, but it induces an increase in GH secretion, which in turn appears to cause the IGF-I peak during puberty (83,84). Oestrogen in high doses reduces IGF-I activity in the serum (85,86). In human, IGF-I levels are normal to slightly decreased in hypothyroidism and increase with thyroxin therapy (87). It appears that T3 modulates the effect of GH on liver production of IGF-I (88). Though it is possible that insulin stimulates IGF-I production in the foetus, it is not likely to be of major influence in postnatal life. Children with poorly controlled insulin-dependent diabetes have low IGF-I concentrations and insulin infusion increased serum levels of IGF-I (89,90). In obese children with hyperphagia normal or elevated insulin levels appear to sustain

IGF-I activity in the presence of low GH secretion (91). However, in hyperinsulinism no increase in IGF-I has been observed (92). Glucocorticoids appear to inhibit in vitro the expression of IGF-I (93). Nutrition has impressive effect on IGF-I synthesis, marked reduction of IGF-I in the circulation is seen in both acute and chronic undernutrition, presumably by a postreceptor blockade of GH effect (53,94).

Biological effects of the IGFs:

Insulin-like growth factors have the capacity to stimulate cell division as well as formation of matrix in cartilage. IGF-I is a growth stimulator similar to GH in mice, rat and humans. Chondrocytes, kidney, spleen and thymus are extremely sensitive to the effects of IGF-I but also all other tissues studied, with the exception of subcutaneous tissue, are stimulated (81, 95-99). IGF-I has a 3-fold function as a mediator of the growth promoting action of GH, as a potent mitogenic factor and as a metabolic regulator with insulin-like activity (95-101).

The possibility that in addition to having an indirect effect through IGFs, GH also has a direct effect on growth of tissues was investigated by Isaksson (98). He showed that GH, when injected in the tibial growth plate of rats, had a local effect. However, locally applied antibodies to IGFs prevented the effect of GH (102). It is proposed that GH induces the differentiation of precursors of prechondrocytes (clone formation), which thereby acquire the capacity to produce IGF-I and respond to it. IGF-I then causes the division of these cells (clonal expansion) by autocrine effect (the dual-effector theory) (103).

GH directly enhances IGF-I production in other tissues as well, though the magnitude of the effect varies. Thus, besides stimulating IGF-I production in liver (which accounts for most IGF-I in blood) and thereby regulating overall growth by the endocrine pathway, GH exerts local effects on tissues that induce increased production of IGF-I, which then will allow paracrine and autocrine growth stimulation (81,104).

The current evidence strongly suggests that IGFs may play a significant role in local regulation of growth in specific organs depending on local needs. For example, mechanical loading at a local site in bone stimulates local bone growth and not generalised skeletal growth. Thus, IGFs may function locally in a variety of cell types; including brain, muscle, kidney, lung, pancreas, and bone, in an autocrine/paracrine

manner in addition to their systemic endocrine actions. IGFs only have two forms that are produced by many tissues. However, tissue-specific regulation may be accomplished partly by the presence of multiple forms of IGF- binding proteins (IGFBPs) and their corresponding proteases (74,105).

The insulin-like growth factor binding proteins:

In the circulation an, about 75 per cent of the IGFs are complexed with IGFBP-3 and an acid labile subunit in a 150- to 200-kd ternary complex, whereas the remainder of the circulating IGFs are bound to lower molecular mass IGFBPs. Six binding proteins have been fully characterised in human (105-107). With the recent development and validation of RIAs for accurate measurements of various IGFBPs in biological fluids, it is now possible to evaluate the relative contributions of all six IGFBPs to the IGF-binding capacity of the serum. The total concentration of the small molecular mass IGFBPs (IGFBP-1, -2, -4, -5, and -6) add up to about 50% of the IGFBP3 concentration. Based on the observation that there is a 50 per cent molar excess of IGFBPs over IGFs, it is unlikely that there is much free IGFs in normal adult human serum (105) .

If the majority of the IGFs circulate in serum as IGF-IGFBP complexes, it should be possible to modulate the endocrine actions of IGF-I by several mechanisms, including those that regulate the levels of IGFBPs. In this regard, there is evidence that the relative concentrations of various IGFBPs in the serum can change depending on physiological and pathological situations, such as age, nutrition, serum GH levels, diabetes, puberty, pregnancy, etc. (108-114). Studies of serum regulation of various IGFBPs to date reveal that the candidate IGFBP chosen as a primary regulator of IGF bioavailability may vary depending on the metabolic conditions. For example, IGFBP3 appears to be the primary regulator of IGF bioavailability in response to changes in the circulating GH level, whereas IGFBP1 appears to be the primary regulator of IGF bioavailability in response to acute changes in the circulating insulin level. In addition, serum levels of IGFBP5, but not IGFBP4, showed significant positive correlation with IGF-I and IGF-II suggesting that different mechanisms may regulate the amount of inhibitory IGFBP4 and stimulatory IGFBP5 in human serum (105)

The stimulatory and inhibitory components of this complex system should be regulated in a reciprocal manner to produce to changes in physiological or pathological

conditions. For example, with age IGFs decrease, the concentration of stimulatory IGFBP5 decrease, whereas the concentrations of inhibitory IGFBP-1, -2 and -4 increase. Collectively this leads to a marked decrease in the ratio of stimulatory IGFBPs to inhibitory IGFBPs, which would tend to decrease the endocrine actions of IGFs (114). Another potential control mechanism for regulation of endocrine IGF action is by IGFBP-specific proteases. For example, IGFBP3 protease has been proposed to play an important role in modulating the bioavailability of IGFs by breaking down the ternary complex ALS-IGFBP3-IGF-I to form the low molecular mass IGF-IGFBP complex, which is capable of crossing the vascular endothelial barrier (105, 115)

IGFBPs as potential modulators of local IGF actions .

In addition to modulating the endocrine actions of IGF as described above, IGFBPs may have other roles including:

a) To increase IGF bioavailability in specific tissue. The proposed mechanisms are (105):
   1. Increase the level of free IGFs.
   2. Increase the ratio of IGFs/inhibitory IGFBPs
   3. Increase the ratio of stimulatory IGFBPs/inhibitory IGFBPs
   4. Increase the rate of proteolysis of inhibitory IGFBPs in the target tissue
   5. Decrease the rate of proteolysis of stimulatory IGFBPs in target tissue
   6. Increase IGF receptor abundance in the target tissue.

b) To facilitate storage of IGFs in extracelluar matrixes. For example data suggest that IGFs are fixed in bone by means of circulating or locally produced IGFBP5, causing high abundance of IGFs in bone (116).

c) To exert IGF-independent effects on target cells. For example, IGFBP3 and IGFBP5 have been shown to mediate their effects on breast cancer cells, and bone cells, respectively, by IGF-independent mechanisms, possibly involving IGFBP-specific cell surface binding sites (117-119). Over-expression of IGFBP3 in fibroblasts derived from IGF-I receptor knockout mouse embryo inhibited cell growth, suggesting that the inhibitory effect of IGFBP3 does not involve signal transduction via type I IGF receptor (120).

IGF receptors:

There are two specific IGF receptors. The type 1 IGF receptor has a structure similar to that of the insulin receptor. It has high affinity for IGF-I and cross-reacts well with IGF-II but weakly with insulin. It possesses tyrosine kinase activity. Type 2 receptor has high affinity for IGF-II and low affinity for IGF-I and does not react with insulin. Both IGF-I and IGF-II have mitogenic effects through interaction with type 1 receptor. The IGF receptors are distributed widely throughout the body (121-123).

B) The hypothalamic-pituitary somatotropic axis during puberty:

The pubertal growth spurt represents the contribution of sex steroids and GH. Each contributes approximately 50% of the height gain: children with GH insufficiency not treated with exogenous GH attain only 50% to 66% of the expected growth spurt (124,125).

Several studies using intermittent sampling techniques have demonstrated a clear rise in GH secretion during puberty (126,127) as well as with respect to pubertal stage (128-130), and this has been supported from studies using the continuous method of sampling (130-132). Growth acceleration in girls takes place during stages 2 and 3 of puberty whereas in boys the growth spurt takes place later in puberty at testicular volume between 10 and 12 ml or genitalia stage 4. Changes in serum GH concentrations parallel these anthropometric changes, and several studies have shown that the change in GH secretion is brought about by an alteration in GH pulse amplitude with detectable GH pulse periodicity remaining unchanged (128-130,133-135). The amplitude of growth hormone peaks increases early in puberty, suggesting that the increase of GH secretion is an amplitude-modulated phenomenon that may be related to either an increase in the responsiveness of the somatotrophs to endogenous GHRH, an increase in GHRH, or a decrease in the tone of somatostatin. Pubertal GH profiles differ significantly from prepubertal profiles not only because of the increased pulse amplitude, but also by the occurrence of that high amplitude GH pulses during the day. In addition, pulse analysis of GH secretion revealed also important changes in the regulation of trough concentrations. Values of the latter increased slightly during puberty but then fell dramatically in early adult life. These findings also suggest that alterations in both GHRH and somatostatin tone take place during childhood and adolescence (43). The majority of the known age-related increase in serum GHBP levels occurs before the period of active pubertal development. These findings strengthen the concept that the midpubertal changes in GH secretion serve a primary role in generating the growth spurt.

Few studies follow the induction of puberty using short courses of testosterone (6 weeks). They reported increased GH secretion and increase in GH pulse amplitude (57,136). Induction of puberty using the hypothalamic peptide gonadotropin releasing hormone (GnRH) was accompanied by changes in GH pulse amplitude but not the

pulse frequency (137-139). Data from a longitudinal study suggest that serum testosterone concentrations need to be elevated toward the normal adult male range for a considerable proportion of the 24-hour period before an increase in serum GH concentration can be recorded (139). Rat studies provide some evidence that oestrogen is a better modulator of GH gene transcription than testosterone, which might require amortisation for its full action (140). In children with gonadotropin-dependent precocious puberty, GnRH agonists are effective in switching off gonadotropin secretion, and thereby sex-steroid concentrations are reduced. With this reduction in sex-steroid concentration there is a decrease in GH secretion and serum IGF-I concentrations (137,138,141).

Changes in the serum concentration of IGF-I in early puberty generally parallel the changes in GH. Early studies demonstrated an increase in IGF-I levels during the pubertal period, but no clear relationship with growth velocity emerged (142,143). Longitudinal studies, show clearly however, that IGF-I levels increase beyond the pubertal growth spurt when height velocity decreases (144). The results do not seem to parallel the GH changes. The interpretation of serum levels of IGF-I is complicated both by its documented role as a paracrine hormone in modulation of growth as well as by the demonstration of multiple IGFBPs modulating its actions (39). A strong negative relationship exists between plasma insulin and IGFBP1 throughout childhood, puberty and adult life (145). These findings suggest that changes in serum IGF-I concentrations during puberty result from the interaction of a number of endocrine axes that evolve at different rates during adolescence and that radioimmunoassay measurement of total IGF-I may not convey the whole story.

The peak velocity of puberty results from the combined action of sex steroids, GH, IGF-I and probably insulin. The final adult height will depend mainly on the age at which puberty is initiated more than on the pubertal growth spurt itself. The earlier the onset of puberty, the more stunting can be expected. Height gain at puberty will thus result from two parameters of growth, namely, height velocity and the duration of growth, both of which will influence final height (146).

Investigating the hypothalamic-pituitary somatotropic axis in growth delayed children:

While the absolute clinical criteria for considering a diagnosis of growth hormone deficiency (GHD) may be somewhat arbitrary the following guidelines are suggested (147,148):

1. **severe growth retardation (height > 3 standard deviations (SD) below the mean for age in the absence of an alternative explanation;**
2. **moderate growth retardation (height -2 to -3 SD below the mean for age plus growth deceleration (height velocity <25th percentile for age) in the absence of an alternative explanation;**
3. **severe growth deceleration (height velocity <25th percentile for age) in the absence of an alternative explanation;**
4. **pubertal delay: in certain situations it may be difficult to ascertain whether a child who is growing slowly with constitutional delay of growth and puberty is going to have an adequate pubertal growth spurt. In such a situation, it may be valuable to perform a growth hormone provocation test following "priming" with exogenous sex steroid before the start of the investigation. The aim of this is to ascertain whether there is going to be sufficient GH available during the puberty growth spurt.**
5. **a predisposing condition (e.g. cranial irradiation) plus growth deceleration;**
6. **other evidence of pituitary dysfunction (e.g. other pituitary deficiencies, neonatal hypoglycaemia, microphalus).**

The pulsatile nature of GH secretion renders assessment of random serum GH concentrations virtually worthless in the diagnosis of GHD. Instead, the convention for over 30 years has been to measure serum GH following pharmacological stimulation of the pituitary, an assessment, presumably, of primary GH "reserve" or secretory capability" (149). While such provocative testing is of value, particularly in identification of patients with complete or severe GHD, total reliance on these tests has proven to be problematic for a variety of reasons (150).

a) **Provocation testing is, by its very nature, non-physiological. Whether such tests employ insulin-induced hypoglycaemia, arginine, L-DOPA, clonidine, glucagon, or other secretagogues, such tests clearly do not replicate normal secretory dynamics.**

b) **No satisfactory mechanism has been developed for resolving conflicting data from two or more tests.**

c) The definition of what constitutes an abnormal response to provocative testing is arbitrary. The cut-off point changed from 5-7 ng/ml before the mid-1980s to 10 ng/ml after the availability of recombinant DNA-derived human GH, on the basis of no physiological data.

d) The age-dependency and sex steroid-dependency of GH secretory dynamics have not been established adequately. The studies of Martin et al (151), shown that in the absence of sex-steroid priming, the lower normal limit for peak GH in prepubertal children is as low as 1.9 ng/ml. Sixty one percent of normal-stature prepubertal children failed to raise their GH concentrations above 7 ng/ml following provocative stimulation and would, thereby, have met the conventional criteria for a diagnosis of GHD.

e) The reproducibility of provocative GH testing has never been demonstrated convincingly.

f) The impact of adiposity, and psychiatric disturbances (e.g. depression) on responsiveness to GH provocative testing has not been addressed adequately.

g) Inter-assay variations in GH radioimmunoassays can be as great as 2- to 3-fold among major reference laboratories.

h) Provocative GH testing is associated with significant cost, discomfort to the patient, and some element of risk. Death has occurred during both insulin and arginine stimulation tests.

i) Demonstration of normal provocation testing does not exclude the possibility of various forms of GH insensitivity (GHI) (152).

j) If it is accepted that growth rate is the most important index (standard) against which growth hormone measurements should be compared, then all the GH provocative tests have different efficiency, sensitivity and specificity. There is little to choose between them (147). At a cut point of GH peak = 15 mU/l (7.5 ng/ml) insulin tolerance test has only 67 per cent efficiency, 69 per cent sensitivity and 63 per cent specificity for diagnosing GHD. The performance characteristics of different tests of GH secretory status compared with the insulin tolerance test using a cut off point value of 7.5 ng/ml (15 mU/l) (147):

Test	Efficiency (%)	Sensitivity (%)	Specificity (%)
Sleep	79	67	82
Clonidine	70	70	85
Arginine	72	73	82

h) A raised GH concentration at time 0 during the test leads to difficulty in interpretation. Tests are designed to evaluate the secretory pathway, particularly the readily releasable pool of GH. If there has recently been a pulse of growth hormone and the pool is low the subsequent response will be attenuated (153).

Despite all the constraints of the tests the likelihood that a slowly growing child is GH insufficient is increased twofold in the presence of a positive test but only decreased by half in the presence of a negative one (147). These serious limitations of the conventional methodology for establishing a diagnosis of GHD have led to the proposal that more useful diagnostic tests would correct these deficiencies. Unfortunately; these deficiencies of the provocative tests have not been corrected by the use of prolonged repeated or continuous sampling of serum for GH (154), or by 24-hour urinary GH excretion (155), measurement of metabolic responses to short-term administration of GH (156), or assessment of the growth responses to GH (157,158).

A proposal that a more useful diagnostic paradigm would be the diagnosis of IGF-I deficiency appeared reasonable (159). The potential advantages of such an approach are: 1) it is clear that IGFs are the major hormones responsible for both intrauterine and postpartum growth, 2) serum concentrations of the critical GH-dependent peptides namely, IGF-I, IGFBP3, and the acid-labile subunit (ALS) have little, if any, diurnal variation and can be readily assessed in a single, random blood sample; 3) radioimmunoassays for IGFBP3 can be readily performed on unextracted samples and are highly reproducible; 4) normal serum concentrations of IGFBP3 are in the μg/ml range, and assay sensitivity is not an issue, and 5) documentation of IGF-I deficiency can then lead to a logical differential diagnosis including:

1. GHD due to hypothalamic dysfunction;
2. GHD due to pituitary dysfunction;
3. GH insensitivity (GHI), either primary or secondary;
4. Primary defects of IGF synthesis or
5. Primary defects of IGF transport/clearance (148).

Measurement of IGF-I and IGFBP3 offers 75 and 96 per cent efficiency and 95 and 97 per cent sensitivity in the assessment of GH secretory status (113,147). Smith et al (158), support the concept that GH secretory capacity in short children covers a continuum. In children who are markedly GH deficient, GH, IGF-I and IGFBP3 are consistently low, and IGFBP2 is elevated. At the other end of the spectrum are short children who have normal GH secretion and normal serum concentrations of IGF-I, IGFBP3 and IGFBP2. Between these two groups are short children in whom GH secretion is marginal or in whom other problems, such as inadequate nutrition, may lead to suboptimal responsiveness. Patients of this type have variable and indeterminate GH secretory responses and may exhibit IGF-I, IGFBP3, and IGFBP2 values that are difficult to interpret and are discordant from GH and each other. On the other hand, when the results of measurements of IGF-I, IGFBP3 and IGFBP2/IGF-I ratio are concordant (as they are in a large percentage of short children), they are likely to provide further understanding of a possible abnormality of GH secretion or action that is not available from measurement of GH alone.

In summary; there is no completely reliable test for diagnosing or excluding GHD in all short children. Also, none of the tests, when used alone, is superior in specificity or sensitivity for making a diagnosis. The use of IGFBP2 and -3 measurements, however, in conjunction with IGF-I can add confidence to the diagnosis when the results are in agreement, even when they do not agree with the GH results. Analysis of the results of the all four tests , particularly when this is performed in conjunction with clinical findings, give the greatest accuracy in diagnosing GHD (158).

Skeletal development and bone turnover: relation to puberty.

Most organ tissues, including skeleton, go through the developmental stages from foetal life to young adulthood. The most critical period in skeletal development is during the time of the most rapid bone modelling and turnover of the adolescence. The process of bone modelling that takes place from birth until the cessation of longitudinal bone growth is characterised by changes in the volume and the shape of the bones. Thereafter, bone tissue within the existing skeletal structure is continuously being formed and resorbed with minimal change in bone size through the remodelling process. From infancy through late adolescence the activity of bone formation predominates, resulting in a steady accumulation of bone mass. On average, most of the skeletal mass is accumulated by the age of 18 (158-161). Thereafter, there is a minimal change in bone mass and density with age up to the time of menopause. Some skeletal sites begin to lose bone immediately after the age of 18 (proximal femur, trabecular bone in the vertebrae), and the other sites show continuous apposition of bone up to the time of menopause (forearm, total spine) (160-162).

Since bone mass is one of the main determinants of fracture, high bone mass at skeletal maturity (peak bone mass) is considered the best protection against age-related bone loss (163). Small differences in bone mass and bone mineral density at maturity of 5-10 per cent could contribute to substantial differences in the incidence of osteoporotic fractures (164).

Bone mass gain during the second decade of life is non-linear and is linked to skeletal age and sexual maturation more so than chronological age. When the influence of chronological age, skeletal age, pubertal stage, and the basic anthropometry on the bone mass and bone mineral density of the total body, forearm, and the second metacarpal bone of females in early puberty have been evaluated simultaneously, of all the independent variables, weight, height, and skeletal age emerged as the most significant predictors for each of the bone mass parameters (160). When relating bone mass to pubertal developmental stage, it becomes obvious that most of the bone mass (37 per cent) is being accumulated between pubertal stage 2 and 4 (160). This rapid accumulation of bone mass correlates with the rate of the longitudinal bone growth and periosteal envelope expansion.

Bone modelling and skeletal consolidation probably result from a complex sequence of hormonal changes in interaction with nutritional factors, where the concerted actions of GH, IGFs, and sex hormones and their receptors are responsible for timing of the bone modelling process and attainment of skeletal consolidation. The increase in circulating IGF-I at puberty correlates with sexual development and results from the interaction between oestrogen and GH. Specifically, the surge in sex steroids in turn increases the secretion of GH, which stimulates the production of IGF-I (165,166). The amount of oestrogen required to stimulate longitudinal growth of long bones is very small. Doses of approximately 100ng/kg/day produce maximal growth in agonadal individuals. These doses seem to be insufficient to cause either the development of secondary sexual characteristics or an increase in sex hormone binding globulin (167,168). These low-dose effects are consistent with the observation that girls attain peak height velocity early in puberty at serum Oestradiol levels of <30 pg/ml, which is one fourth the mean level of adult women (169). During this phase of rapid skeletal modelling (peak growth), which is probably dependent on IGF-I, bone mass is not yet consolidated, and bone mass per bone volume ratio is relatively low. Bone consolidation through endosteal apposition proceeds by the cessation of longitudinal bone growth. This coincides with the increase in Oestradiol secretion by the beginning of menarche and thereafter. It seems, therefore, that oestrogens play an important role in the timing of the growth spurt, stabilisation of bone modelling process, and skeletal consolidation (160).

In a preliminary analysis of bone mass change during puberty in 187 young females (age 11-13.5 years), there was an average of about 264 g of calcium accumulated, which requires a positive calcium balance of about 289 mg/day (164). Abrams et al. (170) measured skeletal calcium kinetics parameters by the use of stable calcium isotope in young females. They found that maximal bone calcium accretion rate is in the pre- and peri-menarcheal period, with the decline thereafter. This should coincide with the peak height velocity or a maximal bone modelling rate in young females. This accumulation of calcium is approximately 25-38 per cent of adult peak bone mass or the whole body calcium for a reference woman, indicating that puberty leads to a dramatic increase in bone mass and correspondingly high skeletal calcium retention, thus presenting the ideal period for dietary manipulation and primary prevention of osteoporosis (161).

Anabolic hormones in bone:

Bone turnover, the product of bone resorption and formation, is a tightly coupled process, with the net balance between the two determining the bone mass and the serum calcium level (171). Regulation of bone turn-over requires the input of a large number of hormones, growth factors, and cytokines (171). An anabolic agent is one that can shift the net balance in the direction of increased bone mass. Additionally, an antiresorptive agent can increase bone mass if it shifts the regulatory balance toward net formation by suppressing resorption. It gradually became clear that a large number of factors interact at the level of the osteoblast, osteoclast, and other cells to regulate the balance between net resorption and formation. Some of these factors include (172):

1. Parathormone (PTH)/PTH-related peptide (PTHrP):

PTH is the most important hormone that regulates calcium levels (171). In addition to its resorptive actions, intermittent administration of PTH produces anabolic effects (173). PTHrP mediates terminal differentiation and function of bone and other tissues. The interplay between physiological levels of PTH/PTHrP and the common receptor, in the osteoblasts, in homeostatic control of serum calcium levels and bone mineral density is essential to long term maintenance of bone structure and function (172).

2.Vitamin D: (1-25-dihydroxyvitamin D3):

Is the key hormone that regulates intestinal calcium absorption. It is a primary resorbing factor in bone through its ability to stimulate the differentiation of undifferentiated bone cell precursor (174). It has effects on the osteoblasts, including regulation of the expansion of extra-cellular matrix proteins such as osteocalcin (175). Vitamin D alone or with calcium supplementation can increase bone mineral density in patients with osteoporosis (176).

3. Androgens: During puberty in boys, administration of androgens

accelerates the accumulation of calcium in bone, consistent with enhanced mineralisation (177), and in elderly or hypogonadal men, administration of androgen has a positive effect on bone mass (178). Human osteoblast-like cells respond to adrenal androgens with changes in gene expression consistent with stimulation of bone formation (172,179).

4. Oestrogen (E2)/ progesterone: Loss of cycling hormones at the menopause results in rapid and significant loss of bone mineral. Although E2 appears to act on osteoclast-like cells to inhibit bone resorption (176). Major attention has focused on the role of E2 in regulating the behaviour of the osteoblast. E2 has an antiresorptive effect on osteoclasts and alters the release of cytokines from osteoblasts and/or mononuclear cells involved in remodelling. Cytokines, such as interleukin-1 (IL-1) or IL-6, which recruit osteoclasts, are suppressed, whereas possible anabolic growth factors, such as IGF-I are stimulated by E2 (180). E2 deficiency also interferes with fibronectin production from osteoblasts, a key element of the extracellular matrix recruitment (181).

5. GH/IGF-I: The production of IGF-I by osteoblasts has led to speculation that IGF-I has both systemic and autocrine actions in bone (182). The effects of IGF-I on bone are anabolic, stimulating longitudinal growth and possibly bone mass (183,184). Gene knockout techniques have shown that mice homozygous for a null IGF-I allele have a defect of ossification, are small and die at birth (185). IGF-I interacts with other bone-active hormones, including PTH and E2, with the former increasing the synthesis of IGF-I (183,186), perhaps accounting for some anabolic effects of PTH on bone (183). IGF-I administration to patients with GH resistance due to mutation in the GH receptor has resulted in increased lean body mass and bone mineral. IGFs affect primarily the differentiated function of the osteoblast with modest mitogenic effects. The ability of IGFs to regulate bone turnover and cause a net anabolic effect on bone is governed by a host of tissue- and cell-specific interactions, including interactions among other growth factors, systemic hormones, extracellular matrix, IGFBPs and different proteases(183,187).

Growth and pubertal maturation in patients with β thalassaemia and sickle cell disease.

A) β thalassaemia :

Growth retardation occurs almost invariably in homozygous β thalassaemia. Significant size retardation is observed in stature, sitting height, weight, biacromial (shoulder) and bicristal (iliac crest) breadths. After the age of 4 years the longitudinal growth patterns display rates consistently behind those of normal controls. The bone age is frequently delayed after the age of 6-7 years. Before the use of chelation therapy, the incidence of normal pubertal development was low. Growth retardation becomes markedly severe with the failure of the pubertal growth spurt (1,188-192).

With the introduction of high transfusion regimes and efficient iron chelation prepubertal linear growth has been improved markedly (192-194). However, abnormal growth is still observed in the majority of patients during late childhood and adolescence (193-195). Borgna-Pignatti et al (193) evaluated growth and sexual development of 250 well- transfused and properly chelated adolescents with β thalassaemia major. Thirty seven percent of their patients were 2 SD below the mean for normal height; after the age of 14 years the percentage was 62 per cent for males and 35 per cent for females. Eighty-three percent of males and 75% of females had delayed skeletal maturation. Complete lack of pubescent changes was present in 38 per cent of females and 67 per cent of males aged 12 to 18 years. Only 19 per cent of females had experienced menarche; secondary amenorrhoea intervened in a third of them. Recently a multi-centre study, performed in 25 Italian centres, surveyed 1861 patients with thalassaemia major on frequent blood transfusion and iron chelation. The study showed that failure of puberty was the major clinical endocrine problem and was present in 51 per cent of boys and 47 per cent of girls, all over the age of 15 years. Secondary amenorrhoea was recorded in 23 per cent of patients (mean age 18.3 years) . Another cross-sectional study in Hong Kong, performed on 35 girls and 33 boys with thalassaemia major, revealed that despite regular transfusion and chelation therapy, 75 per cent of the girls and 62 per cent of the boys over the age of 12 years were below the 3rd percentile for height. Hypogonadism was found in a similar percentage of patients (196).

Bronspiegel-Weintrob N et al (197) reported that beginning chelation treatment with deferoxamine well before puberty can help children with transfusion-dependent thalassaemia major to attain normal sexual maturation. However, the benefits of early, intensive therapy with deferoxamine on sexual maturation must be weighed against the reports of deferoxamine-induced growth failure and bony changes in patients treated in the first 14 months of life (197,198). It is observed that in the past some of the growth retardation of thalassaemic children may have been due to the effect of severe anaemia and hypersplenism from early life. In adequately transfused patients linear growth retardation occurs during late childhood and adolescence, the basis for this distressing symptom remains unclear (1).

B) Sickle cell disease:

Growth and maturational delay are striking features of sickle cell disease. Abnormalities include early deficit in height and weight and delay in skeletal maturation. The majority of patients have low weight-for-height and height-for-age parameters (199-203). Studies using cross-sectional data showed that the onset of menarche and pubertal development are also delayed (201-206), but puberty appears to progress normally (207). A longitudinal controlled study (208) performed on Jamaican cohort with sickle cell disease proved that the onset of the adolescent growth spurt was delayed in sickle cell disease by 1.4 years with no sex difference. The age at menarche in girls (15.4 +/- 1.3 years) was significantly later than those with normal HbA (13.1 +/- 1.3 years). They had a characteristically reduced spinal (sitting) to subischial length at the age of 16 years. The age at peak height velocity was delayed by 1.6 year compared to normal controls. However, the adolescent growth of these patients was otherwise normal. This study suggested a normal but delayed pattern of adolescent growth in most children with sickle cell disease but failed to explain the extreme growth delay found in a subgroup of patients with SCD.

In this study we evaluated the auxological data of two cohorts of children with thalassaemia and sickle cell disease including age, weight, height (Ht), Ht SDS, body mass index (BMI), upper/lower segment ratio and annual growth velocity (cm/year) and compared them to those for normal age-matched normal children and a cohort of normal children with constitutional short stature. In addition the pubertal stage was assessed (breast development in girls and testicular volume in boys) in all the patients

older than 9 years of age. Skeletal maturation was assessed in these children by estimation of the bone age of the left hand and wrist plain-x-rays.

AETIOLOGY OF GROWTH RETARDATION AND PUBERTAL ABNORMALITIES IN β THALASSAEMIA AND SCD:

The reason for growth retardation and pubertal delay in these two forms of chronic haemolytic anaemias has not been clearly determined. Haemosiderosis- induced damage of the endocrine glands is implicated to be one of the main causes for their growth failure (195-208). However, other factors could considerably contribute in the aetiology of this growth delay including 1. the effect of chronic anaemic hypoxia secondary to low haemoglobin concentration; 2. toxicity of desferrioxamine treatment; 3. increased energy expenditure due to high erythopoietic turn-over and cardiac work; 4. nutritional deficiencies including calories, folic-acid, zinc, and vitamin A; 5. disturbed calcium homeostasis and 6. hepatic and pancreatic dysfunction (209-220).

Hepatic involvement in thalassaemia and sickle cell disease:

Serial liver biopsies from thalassaemic children from early in life show evidence of iron loading by both light and electron microscopy. It appears that iron loading and tissue damage occurs remarkably early in life. Most of the hepatocytes, especially after two years of age, contain large number of round or oval structures, single-membrane bound lysosomes (variable amount of ferritin and haemosiderin or both), with increased total iron content. Ferritin molecules are seen in association with lamellae, either in paracrystalline configuration or without specific arrangement. Early in life there is no morphological evidence of subcellular damage or change of hepatic architecture. In material obtained from older children and adolescents there is advanced cirrhosis with many hepatocytes and Kupfur cells appear totally filled with iron-laden lysosomes. Later on, there are excessive numbers of collagen fibres. These findings suggest progressive liver damage from very early life in children with thalassaemia. Additionally, as a result of repeated blood transfusion, these children are at higher risk of developing hepatitis. Surveys in the Mediterranean region and Southeast Asia showed high prevalence of hepatitis- B antigenaemia in patients with thalassaemia. It is possible that in a number of patients progressive damage and fibrosis results from effects of viral hepatitis in addition to the iron overload (1,195-197,218,219).

In this study we evaluated the different hepatic functions in 110 patients with sickle cell disease, 70 patients with thalassaemia and 100 normal age-matched children, and studied the prevalence of persistent hepatitis-B surface antigenaemia and hepatitis-C virus (HCV) antibody in these children. We also studied the prevalence of hepatitis-C virus (HCV) antibody and different hepatic functions in a randomly selected sample of healthy children, a group of thalassaemic children and in three other high risk groups.

The nutritional status of these patients were evaluated by measuring their auxological parameters including BMI, mid-arm circumference, and subcutaneous fat thickness at three different sites (biceps, triceps and scapular skin fold thickness). Their food intake was evaluated using the recall method for the last three days. Seventy two-hour faecal fat content was measured in two randomly selected groups of patients with thalassaemia and sickle cell disease and D-xylose test was performed to evaluate their exocrine pancreatic function and intestinal absorption respectively.

Endocrine dysfunction in thalassaemia and sickle cell disease:

As a part of iron loading of the tissues which occurs in homozygous β thalassaemia there is widespread haemosiderosis involving most of the endocrine organs. Thalassaemic patients suffer from a variety of endocrine deficiencies, particularly as they reach the second decade when iron loading becomes marked. Several recent studies analysed the different endocrine function in thalassaemic patients. These studies confirm that there is widespread endocrine dysfunction by the time heavily transfused thalassaemic children reach the second decade (194-197,221).

1. Impaired glucose tolerance and diabetes mellitus in thalassaemia:

Probably the commonest endocrine abnormality is glucose intolerance and diabetes mellitus. The reported prevalence of diabetes in treated thalassaemic patients is about 16 per cent, while the incidence of impaired glucose tolerance approximates 60 per cent (222-223). It appears that the duration of the disease and the number of transfusions are highly correlated with the degree of glucose intolerance (223-227). Possible pathogenic conditions are progressive pancreatic cell destruction with subsequent insulin deficiency(224-228), liver derangement with consequent insulin resistance (229-

231), genetic factors(223,227), and possible immunological disturbance. In addition, 28 per cent of these patients develop diabetes mellitus shortly after an acute viral hepatitis infection, suggesting an important role of hepatitis virus in precipitating a diabetic state in these patients (232). Data about the circulating concentrations of insulin in non-diabetic children with thalassaemia are controversial. Toccafondi R et al (233) reported lower insulin response to oral glucose in their thalassaemic children with normal oral glucose tolerance (OGTT) and Lassman et al (224,234) found a delayed insulin response to oral glucose loading. In controversy Flynn et al (226) and Merkel (235) and others (236,237) described high insulin level in patients with normal OGTT denoting insulin-resistance. Two studies suggested that hyperinsulinaemia is mainly due to decreased hepatic insulin extraction (236,237). However, in one of these studies, over time patients with thalassaemia experienced a reduction in their circulating insulin levels (237) .Kuo et al (238) found that in only 50 per cent of older thalassaemic patients did the plasma glucose fall to less than half of the fasting levels after intravenous (iv) insulin, whereas Toccafondi et al (239) found that plasma glucose fell normally after iv insulin in thalassaemic children under 10 years.

Diabetic hyperglycaemia is generally believed to be a bihormonal disorder where absolute or relative lack of insulin and excess of glucagon cause decreased peripheral glucose uptake and increased hepatic glucose production (240-244). In agreement with this notion, increased hepatic glucose production and plasma glucagon concentrations have been demonstrated to correlate with the hyperglycaemic level (241-247).

Lassman et al (224) found diminished glucagon response to intravenous alanine infusion in thalassaemic patients without family history of diabetes and suggested that this is due to iron loading of the alpha cells. In controversy, Passariello and Nelson et al (248,249) demonstrated elevated glucagon level in thalassaemic and haemochromatic patients.

In this study we prospectively evaluated insulin and glucagon secretion in response to oral glucose load and arginine infusion in young thalassaemic children before and after long-term high transfusion and chelation therapy.

2. The hypothalamic-pituitary somatotropic axis in thalassaemia and sickle cell disease:

Thalassaemia major:

GH secretion has been studied by different authors in thalassaemic patients with growth delay. Results are still controversial, both normal (250,251) and subnormal GH response (252-254) to different provocative stimuli have been reported in these patients. Studying the spontaneous pulsatile secretion in thalassaemic patients with normal GH response to provocation also showed different results by different authors. Some authors reported neurosecretory dysfunction of GH secretion in the majority of their patients (252,255), Others reported dysfunction only in very few subjects of their studied cohort (256). In controversy, Pasqualetti et al found normal circadian secretory pattern of GH in their patients (257).

In contrast to GH results, circulating IGF-I concentrations have been always reduced in all the studies (258-259). Alteration of IGF-I regulation may provide an attractive explanation for thalassaemia -associated growth impairment. The predominant insulin-like growth factor binding proteins IGFBPs in the blood is IGFBP3, which forms a large 150-kilodalton ternary complex. The serum level of this complex determines the total concentration of circulating IGF-I and regulates its growth promoting potential (105-107). Current opinion favours GH as the major regulator of IGF-I and IGFBP3 levels in humans. In addition, serum IGF-I and IGFBP3 are positively related to nutritional status and affected by other hormones like insulin. Estimation of circulating concentrations of IGF-I and IGFBP3 markedly improves the interpretation of GH data in response to provocation and allows better evaluation of the hypothalamic-pituitary growth axis (113,147,158).

Sickle cell disease:

The basal circulating concentrations of different hormones have been studied by different authors (260-262), with no consensus defining the endocrine abnormalities of their GH/IGF-I growth axis and their possible contribution to growth impairment in these children.

This study was conducted to : 1. investigate the GH/IGF-I/IGFBP3 axis in prepubertal children with beta thalassaemia major and sickle cell disease. This included studying GH response to provocation by clonidine and glucagon, evaluation of spontaneous nocturnal GH secretion in thalassaemic patients who had normal GH release after provocation, and estimation of circulating levels of IGF-I and IGFBP3 in these patients; 2. test the hypothesis that these patients might have GH resistance by applying the IGF-

I generation test; and 3. study the effect of GH therapy for one year on their growth parameters.

3. The hypothalamic-pituitary gonadal axis in thalassaemia and sickle cell disease:

Studies of gonadotropin levels have given inconsistent results. Lassman et al (263) found low luteinising hormone (LH) levels in their post-pubertal subjects who also showed clinical hypogonadism and a low concentration of oestrogen and testosterone in the blood. This is in contrast to the findings of Flynn et al (226) where there was a tendency for LH but not follicle stimulating hormone (FSH) to be raised in both boys and girls in relation to their stage of puberty. Tato et al and Danesi et al (264,265), reported reduced LH and FSH rise in response to gonadotropin releasing hormone (GnRH). Chatterjee et al (266) found low-normal GnRH-stimulated gonadotropin levels in 15 thalassaemic girls who developed secondary amenorrhoea. Studying the spontaneous pulsatile properties of LH and FSH in those thalassaemic girls revealed progressive neurosecretory dysfunction of their gonadotrophins (266). However, pulsatile GnRH treatment of boys with thalassaemia and failure of puberty resulted only in partial correction of the pituitary-gonadal function (267). In females, sequential treatment with human menopausal gonadotropin (HMG) followed by human chorionic gonadotropin (HCG) induced ovulation and increased Oestradiol secretion in some hypogonadal thalassaemic women (265). HCG administration to hypogonadal boys with thalassaemia induced and maintained secondary sexual characteristics in some of them (268). On the other hand, testicular autopsies revealed varying degrees of testicular interstitial fibrosis with small, heavily pig undifferentiated semineferous tubules, hyalinised slings and an absence of Leydig cells suggesting end-organ fibrosis secondary to iron loading (269).

4. Other endocrinopathies in thalassaemia:

- *Adrenal function* studies in thalassaemic patients have also given inconsistent results. Many studies reported normal 8.00 AM serum cortisol concentration, normal urinary hydroxycorticosteroid levels and normal cortisol response to adrenocorticotrophic (ACTH) in thalassaemic patients (225,238,269,270). Basal concentrations of other adrenal cortical hormones e.g. andrestenidione and dehydroepiandrosterone, were found to be normal in one study (270). However, other

investigators found low basal 17-ketosteroid levels and poor response to metyrapone (238), reduced cortisol response to tetracosactrin , and elevated early morning plasma ACTH suggesting end-organ unresponsiveness (225).

Mild abnormalities of *thyroid function* have been found in some patients with β thalassaemia with variable, though low, incidence of thyroid failure (226,234,270,271).

***Parathyroid* function may be diminished in early childhood and clinical hypoparathyroidism has been documented in a few cases (226,271,272)**
In this study we measured 8:00 A.M. serum cortisol, thyroxin (T4) and thyrotropin (TSH) concentrations and performed low-dose and high-dose ACTH tests to investigate cortisol reserve in a large cohort of thalassaemic children to investigate their adrenal and thyroid functions. Serum calcium, phosphate, and alkaline phosphatase concentrations were measured to investigate calcium homeostasis in these patients

Skeletal changes in thalassaemia and sickle cell disease:

Beta-thalassaemia major **is associated with significant bone disease. (272) These changes include bone marrow expansion of the medullary cavity, cortical thinning, trabecular coarsening with various striations or the appearance of cystic spaces and coarsening of the bone pattern with drop out of all but the mechanically most necessary trabeculae.(273)**

With advances in transfusion management beginning in the 1996s, there has been marked improvement in skeletal development and normal cosmetic facial and long bone appearance.(274) However, even the well transfused patients remain radiographically osteopenic . Prior to hypertransfusion regimens fractures have occurred in long bones and were associated with trauma. (275) After high transfusion, the pattern changed with less long bone involvement and more vertebral compression fractures especially in older patients (276).

Although the aetiology of the bone disease is still debatable, many factors can adversely affect bone accretion in thalassaemic children. These include: 1. Chronic hypoxaemia and medullary expansion, 2. Defective growth, affecting both height and weight, 3. Abnormal calcium-phosphate homeostasis, 4. Delayed or lack of pubertal development and decreased sex-steroid secretion, 5. Compromised nutritional status and increased energy expenditure, 6. abnormal (GH)/ I (IGF-I)/ (IGFBP3) axis and/or 7. The development of diabetes mellitus (194,215,219,221-223,225,226,233-238)

Sickle cell disease **is associated with many risk factors that can adversely affect bone acceleration. These factors include:1. avascular necrosis during sickling episodes, 2. delayed growth and pubertal development, 3. deficiency of important growth factors (IGF-I), 4. nutritional deficiencies; 5. disturbed calcium metabolism (199-214).**
Many radiological changes are described in these patients which become marked with age. These changes combine two features , namely expansion of the medulla of the long bones and the diploic spaces of the cranial vault and the effect of bone infarction and infection. The cortex and trabeculae may be thinned and the intertrabecular spaces are widened throughout the skeleton. In the small bones of the hands and feet deposition of

periosteal new bone along the shaft is combined with irregular areas of translucency within the substance of the bone. The whole bone may be involved. Interference with bone growth can be generalised or localised to the metaphysis of the long bones and vertebral bodies (273).

Because of the above mentioned risk factors and the significant radiological bone changes in patients with thalassaemia and sickle cell disease, we measured bone mineral density and investigated some factors affecting bone mineral metabolism in relation to linear growth and secretion of growth factors (GH), (IGF-I), and IGFBP3) in 30 children with thalassaemia, 28 prepupertal children with SCD and a group of age-matched normal children.

In summary , patients with thalassaemia and sickle cell disease have high incidence of growth and pubertal delay and secondary dysfunction of their endocrine glands, despite improvements in the care of haematological problems. The aetiology of this delay has not been clearly determined but likely to be multifactorial. Studying the different factors that might adversely affect growth, with early prevention and/or treatment of these factors, is a very essential part in the long-term care of these patients.

OVERVIEW OF THE STUDIES

THE AIM OF THE STUDIES

These studies were conducted to investigate the different factors that might contribute to growth retardation in children with thalassaemia and sickle cell disease and to specifically answer the following questions:

1. **Do children with thalassaemia and sickle cell disease have significant growth retardation and/or pubertal delay?**
2. **Are these patient undernourished? Do they eat qualitatively and quantitatively adequate food?**
3. **Do children with thalassaemia and sickle cell disease have significant impairment of hepatic functions? Is it related to the degree of iron overload and/or transfusion-associated hepatitis?**
4. **Do children with thalassaemia and SCD have abnormalities of GH/IGF-I/IGFBP3 axis?**
5. **Does GH therapy increase linear growth in these patients? Do they have resistance to GH?**
6. **Do thalassaemic children on long-term blood transfusion develop progressive impairment of insulin secretion?**
7. **Do children with thalassaemia and sickle cell disease have abnormalities of the pituitary-thyroid and/ or the pituitary adrenal axis?**
8. **Do children with thalassaemia and sickle cell disease have decreased bone mineral density (osteoporosis)?**

To answer the previous question we performed the following studies (papers 1 through 12):

Study -1: GROWTH AND PUBERTAL DEVELOPMENT IN TRANSFUSION-DEPENDENT CHILDREN AND ADOLESCENTS WITH THALASSAEMIA MAJOR AND SICKLE CELL DISEASE (SCD): A COMPARATIVE STUDY

(Journal of Tropical Paediatrics, Accepted for publication, November 1997)

(Presented in the XXII International Congress of Paediatrics, Amsterdam , 9- 14 August, 1998)

THE AIM OF THE WORK:

1. **to perform a systematic survey of growth and pubertal development in a large cohort of patients with thalassaemia major and sickle cell disease (SCD), all received the same treatment during the last 10 years to investigate the effect of the current regimen on growth and pubertal maturation of these patients.**
2. **to assess the nutritional status of these children by evaluating their diet and measuring their body mass index (BMI), mid-arm circumference (MAC) and subcutaneous fat thickness.**

PATIENTS AND METHODS:

Patients with transfusion-dependent thalassaemia major and SCD were randomly selected from the Haematology Clinics of the Royal Hospital and Alexandria University Children's Hospital, Alexandria, Egypt. The participating hospitals were chosen with consideration of homogeneity of treatment protocol. Mean pre-transfusion haemoglobin (Hb) concentration has been kept at 9 g/dl in the last 7 years in most patients; individual variability was not such as to permit comparisons between patients. They received desferrioxamine (50 mg/kg/dose) by intramuscular and/or subcutaneous injection 3 times weekly. All were on folic -acid supplements and all had been vaccinated against pneumococci. Two hundred age and sex matched normal randomly selected children , 30 children with constitutional delay of growth (CSS) (height = or > 2 standard deviations (SD) below the mean for age and sex, with delayed bone age and normal growth hormone (GH) response to provocation), and 25 children with isolated GH deficiency (GHD) , served as controls. None of the children had history of intrauterine growth retardation, any other systemic or endocrine disease, dysmorphic

trait, or central nervous system irradiation. Informed consent for the testing procedures was obtained from the parents of all children and when appropriate from the children. All children were examined with special emphasis on nutritional data. A special form was prepared for this study. The anthropometric measurements included weight, height, MAC and triceps and sub-scapular skin fold thickness during a hospital visit at the time of the study. Harpenden's callipers and anthropometric measurements were used. The height SDS (HtSDS) was calculated according to the formula HtSDS = (X1 - X2) / SD, where X2 and SD are age matched population mean height and SD, respectively, and X1 is the subject height. The height growth velocity (GV) cm/year was calculated for a complete year, following the first measurement. Normal population data were according to Tanner et al (277). The BMI was calculated according to the formula = weight (Kg) / height (m)2. The bone age and stages of sexual maturation were evaluated according to Greulich and Pyle (278) and Tanner et al (277) respectively. In girls, the age of menarche and early breast development were recorded. In boys, testicular volume was measured and recorded.
Dietary intake, both qualitative and quantitative, was assessed by the dietician for all patients and 50 normal age-matched children using the recall method. A standard oral glucose tolerance test (OGTT) was performed (1.75 g glucose / kg body weight). Three patients with impaired glucose tolerance were excluded from the study.

In a randomly selected sample from short children (height = or > 2 standard deviations (SD) below the mean for age and sex) from each group {thalassaemia (n=15), SCD (n=21) and CSS (n=10) a fasting venous blood sample was obtained and kept frozen at -20 degree C until analysed for thyroxin (FT4), thyrotropin (TSH), and IGF-I concentrations by radio-immunoassay. All samples from all the children were assayed simultaneously.

RESULTS: (see appendix for tables and figures)

The HtSDS , GV and GVSDS of patients with thalassaemia and SCD were significantly decreased compared to normal children ($p<0.01$) (table 1). The HtSDS of thalassaemic children was significantly lower than that for children with SCD. Both groups had HtSDS higher than those with GHD. The GV and GVSDS of patients with thalassaemia were lower than those for SCD, however the difference did not attain statistical significance for the GVSDS ($p=0.09$). Children with thalassaemia and SCD had GV and GVSDS significantly higher than those with GHD but not different than those with

CSS. The bone age delay (yr) did not differ among the three study groups with thalassaemia, SCD and CSS. The BMI of thalassaemic patients was significantly higher compared with the other groups. The mid-arm circumferences were significantly decreased in children with thalassaemia and SCD compared to normal children (table 2). The triceps skin-fold thickness of children with SCD was significantly decreased compared to that for normal children. Assessment of the food intake, using the recall method for the last 3 days, failed to detect any qualitative or quantitative deficiency of food consumption by children with thalassaemia or SCD. The upper segment / lower segment ratio (U/L) in children with thalassaemia (0.85 +/-0.07) and SCD (0.89 +/- 0.09) was significantly lower than age-matched controls (1.09 +/- 0.06).

Figures A (1-3) and B (1-3) and table 3, show the HtSDS, GVSDS, and BMI data of children with thalassaemia and SCD. Linear growth was significantly impaired in both groups of children with thalassaemia and SCD. Forty nine per cent of thalassaemic children and 27 per cent of those with SCD had HtSDS below -2. Eighty three per cent of thalassaemic children and 67per cent of children with SCD had HtSDS below -1. A considerably large percentage of thalassaemic and sickler children had slow growth velocity; 56 per cent of thalassaemic children and 51 per cent of children with SCD had GVSDS below -1 during a full year of linear growth. Figure C (1,2) shows scatter-grams of chronological age versus HtSDS and GVSDS in patients with thalassaemia and SCD respectively. The age was correlated negatively with HtSDS ($r = - 0.405$, $P < 0.01$) suggesting progressive growth retardation with age in these patients. Serum ferritin concentration was correlated negatively with GV ($r = -0.45$, $P < 0.001$) (figure C).

Pubertal development (table 4): If testicular volume = or > 3ml and breast development > B1 are taken as evidence of beginning of pubertal development, a considerable number of patients had not experienced sexual maturation, even at ages when this is the rule in normal subjects. The data of twenty two thalassaemic boys, between the age of 14 and 21 years (16.9 +/- 3.8 years), showed that only 6 (27 per cent) of them had testicular enlargement (volume > 3ml). Their mean testicular volume = 6.8 +/- 2.5 ml. Out of the 19 thalassaemic girls between the age of 13 and 22 years (17.2 +/- 3.2 years) only 5 (26 per cent) had spontaneous menarche at a mean age of 17.5 +/- 1.2 years. Breast development was delayed in 11 (B2 at mean age of 15.7 +/- 1.5 years) and absent in 8 of them. Three out of the five patients who had spontaneous menarche had irregular menstrual cycles and two of the three had secondary amenorrhoea.

In girls with SCD above the age of 13 years (16.5 +/- 3.2) (n=16) , 14 girls had delayed breast development (B2) after the age of 13 years (mean age of 13.5 +/- 0.4 years) and only 2 had breast development before the age of 13 years (B2 at mean age of 12.5 +/- 0.4 years). Seven girls, out of the 14, had spontaneous menarche at an age of 15.6 +/- 0.7 years. Out of the 18 boys with SCD, above the age of 14 years (17.1 +/-2.8 years) , 14 had testicular volume above 3 ml (Tanner 2 or more) and four (25 per cent) had testicular volume < 3 ml at the age of 14, 14.5, 15.5 and 16 years respectively. Their mean testicular volume = 8.5 +/- 3.1. In thirty normal males (age 16.9 +/- 2.6 years) and thirty normal females (age 16.2 +/- 2.3 years) ; between the age of 13 and 21, the mean age for starting breast development (B2) ranged between 9.5 and 13.1 years with a mean = 10.7 +/- 0.8 years. The mean age of menarche was 13.4 +/- 1.2 years. All the normal adolescent boys (n=30, age 16.9 +/- 2.6 years) had testicular volume above 4 ml. Their mean testicular volume = 12.4 +/- 3.4 ml.

Serum ferritin , and alanine- transferase concentrations were significantly higher in thalassaemic children versus the other two groups (table 5). Insulin-like growth factor-I concentration was significantly lower in children with thalassaemia and SCD versus children with CSS. Circulating IGF-I concentrations were significantly lower in children with thalassaemia compared to those with SCD. FT4 and TSH concentrations did not differ among the three study groups.

DISCUSSION:

Subjects from the cohort study were diagnosed either at birth or during infancy; are a representative sample of children with thalassaemia and SCD. Analysis of cross-sectional and longitudinally collected auxological data and comparison with normal children as well as with 2 groups of children with CSS and isolated GHD allowed better evaluation of growth in these patients. The present data prove the high prevalence of short stature in children with thalassaemia and SCD during childhood and adolescence. This was more marked in thalassaemia (about half of the thalassaemic children had HtSDS below -2). In addition the linear GV of more than half of the children with thalassaemia and SCD was slow (GVSDS below -1). Again this slow GV was more marked in thalassaemic children than in SCD. The HtSDS and GVSDS of children with thalassaemia and SCD were comparable to those for children with CSS and significantly higher compared to children with GHD. The skeletal age, as well, was

more delayed in children with GHD versus those with thalassaemia and SCD. Collectively these data, in accord with the results of other investigators, (188-196) confirmed delay in linear growth in children with thalassaemia and SCD. The significant (negative) correlation between the chronological age on the one hand and the HtSDS and GVSDS on the other hand pointed out to the fact that linear growth delay progressively increases with age in these patients.

Patients with thalassaemia and SCD had significantly decreased mid-arm muscle circumference, and those with SCD had decreased subcutaneous fat thickness. In these patients undernutrition; might be due to decreased food intake , a hypermetabolic state (209) due to increased bone marrow activity, and/or deficiency of the anabolic hormones e.g. IGF-I. The higher BMI in patients with thalassaemia does not accurately reflect the nutritional status because all of them had significant hepatomegaly with or without splenomegaly disturbing the weight to height ratio. Dietary intake of these patients appeared to be qualitatively and quantitatively adequate suggesting that probably other factors, such as hypermetabolism (209) and/or defective intestinal absorption of nutrients (217), might be working.

Delayed onset of puberty was a frequent finding in both boys and girls with thalassaemia and SCD. The delay/failure of puberty was more pronounced in patients with thalassaemia ; with more than 70 per cent of girls were suffering from primary or secondary amenorrhoea and more than 70 per cent of boys older than 14 years had delayed testicular development. In these patients the significantly low U/L segment ratio reflected their hypogonadal status but can be also due to vertebral changes secondary to hyperactivity of the bone marrow.
The aetiology of retarded growth in children with thalassaemia and SCD is probably multi-factorial with contributions from abnormal endocrine function,(194-197,221-249) suboptimal nutrition (210,215,217), an increase in metabolism due to hyperactivity of the bone marrow (209,217) and hypogonadism (193,195,196). In this study serum IGF-I concentrations were significantly depressed in short children with thalassaemia and SCD compared to short normal children, denoting defective IGF-I synthesis in these patients. This might be due to defective GH secretion (251-255,258,259) and/ or resistance to GH due to liver siderosis (218, -220, 258). The significant (negative) correlation between GV (cm/yr.) and serum ferritin concentrations supports the view of decelerated growth with increasing iron load. Under-nutrition, as shown in our patients by reduced mid-arm circumference and subcutaneous fat thickness, might contribute to

growth delay in these children through decreased IGF-I synthesis. Some investigators report improvement of growth after increasing the caloric intake of these patients (215). Moreover, in many hypermetabolic states there is some degree of GH resistance, which might be a contributing factor in decreased synthesis of IGF-I and consequently delayed growth (280). Delayed and/or failure of puberty occurred in a considerably large numbers of males and females with thalassaemia and SCD. The lack of the synergistic action of sex steroids on pubertal growth spurt appears to be a major factor contributing to impaired growth in these patients. In this study HtSDS and GVSDS were correlated negatively with the chronological age supporting the view that as patients with thalassaemia grow older, without achieving pubertal development, their linear growth becomes more delayed

In summary, marked delay or failure of growth and puberty in children with thalassaemia major and SCD still occur in a large number of patients. This denotes that treatment with the current transfusion and chelation protocol is sub-optimal and necessitates the application of more aggressive program for hyper-transfusion and iron-chelation to improve growth in these patients. Improvement of the nutritional status, by increasing the caloric intake, should be an essential part of any recent protocol for treatment of these patients. Early detection of delayed / failed puberty and proper management by physiological replacement of sex-steroids and/or gonadotrophins should improve their pubertal growth.

Study-2: STUDY OF HEPATIC FUNCTIONS AND PREVALENCE OF HEPATITIS-B SURFACE ANTIGENAEMIA IN OMANI CHILDREN WITH SICKLE CELL DISEASE.

(Journal of Tropical Pediatrics 1995; 41:174-76)

THE AIM OF THE WORK:

To study the hepatic functions and prevalence of persistent hepatitis-B surface antigenaemia and hepatitis-C antibody seropositivity in children with sickle cell disease and study the relation, if any, between the liver function and linear growth in these patients.

PATIENTS AND METHODS:

One-hundred-and-twenty-five children between the ages of 3 and 12 years with SCD were the subject of this study. They represented the sickle cell children in Muscat area attending the Paediatric Haematology Clinic of the Royal Hospital, Muscat, Oman. One-hundred normal age-matched children served as controls. All the study children were subjected to thorough history taking, with special emphasis on previous episodes of jaundice, injections, and blood transfusions. Full clinical examination was performed, and the size of the liver and spleen recorded. The height and weight were measured accurately using Harpenden's measuring instruments and the HtSDS calculated and recorded. Blood samples were collected for estimation of serum alanine transferase (ALT), alkaline phosphatase (ALP), albumin, and bilirubin concentrations. All serum samples of the study children were tested for the presence of hepatitis B surface antigen (HBSAg) using the latex quick test (281), and IgG antibody to hepatitis C virus (HCV) by indirect ELISA test (Behring).

Statistical analyses were done using the t-test for comparison between the different study groups when the data were normally distributed and Wilcoxon test when they were not. Data are presented as mean +/- SD.

RESULTS: (see appendix for tables)

Table 1 shows the hepatic functions of children with SCD and controls. Four out of the 125 children with SCD were HBSAg carriers (3 per cent) and 10 had circulating HCV antibodies (8 per cent), while 11 out of the 100 normal children were HBSAg carriers, and 7 were HCV-antibody positive. Serum bilirubin and ALT concentrations were higher in the sickle group compared to controls. Serum ALT and bilirubin levels were

significantly higher in HBSAg negative sicklers (n=121) than those for HBSAg negative controls (n=89) (table 2). Table 3 compares the liver functions of HBSAg positive sicklers and controls. Serum bilirubin and ALT concentrations were significantly higher and serum albumin levels were markedly lower in sicklers' group. Table 4 presents a comparison between two groups of sickle cell children with and without HBS-antigenaemia. Sicklers with HBS antigenaemia had significantly higher ALT and lower albumin concentrations compared to those without HBS antigenaemia.

Out of six children with SCD who presented with HBSAg positive acute hepatitis, two patients did not clear the antigen from their serum after one year, with elevated serum bilirubin (59 and 85 umol/L) and ALT (87 and 110 IU/L) concentrations. None of the normal children with acute HBSAg positive hepatitis were still carrying the antigen after 3 months of the attack.

The concentrations of ALT were correlated significantly with the height SDS in patients with SCD ($r = -0.21$, $p = 0.026$).

DISCUSSION

This study shows that children with SCD do not have a high prevalence of HBS antigenaemia. Our results confirm the findings from Nigeria that the incidence of hepatitis in sickle cell children is no higher than the incidence in the general population (282).

Significant elevation of serum ALT and bilirubin concentrations in children with SCD compared to control children denotes the presence of mild chronic hepatocellular dysfunction which might be due to liver siderosis, repeated vascular-hypoxic insults and/or chronic viral inflammation. However, this is not related to hepatitis-B virus because it is present also in sickle cell patients without HBS antigenaemia.

Previously normal persons usually clear the HSAg from the serum within 4-6 weeks from the onset of acute symptoms. Chronic hepatitis is associated with persistent antigenaemia (283). In this study, 33 percent of sicklers with acute hepatitis-B infection did not clear the antigen for 1 year whereas, all the normal children cleared the antigen from the serum after 3 months. In addition, the comparison in table 4 proves that in children with sickle cell disease chronic carrying of HSAg is accompanied with more deterioration of hepatic functions (high ALT, and bilirubin concentrations). These findings point to the high risk of chronicity and continual parenchymal damage in sicklers after acute hepatitis-B infections. This can drastically influence the prognosis of

hepatic involvement in this disease. It appears that vaccination against hepatitis-B should be a useful tool to eliminate this risk.

The significant correlation between serum ALT concentrations and height standard deviation score (HtSDS) ($r = -0.21$, $p = 0.026$) in patients with SCD suggests that impairment of hepatic function might adversely affect linear growth in these children.

Study-3: STUDY OF HEPATIC FUNCTION AND PREVALENCE OF HEPATITIS-C ANTIBODY SEROPOSITIVITY IN THALASSAEMIC CHILDREN AND THREE HIGH RISK GROUPS.

(Journal of Tropical Pediatrics 1995;41:341-344)

AIM OF THE STUDY:

To evaluate the hepatic function and estimate the prevalence of hepatitis-C virus antibody in a sample of healthy Egyptian children (n=110) as well as in four high risk groups of children with thalassaemia, insulin-dependent diabetes mellitus (IDDM), schistosomal hepatic fibrosis and chronic rheumatic heart disease.

PATIENTS AND METHODS:

186 children between the age of 1-16 years were the subject of this study. Group(1) included 18 children with thalassaemia major on regular blood transfusion and iron chelation therapy, group (2) included 17 children with insulin-dependent diabetes mellitus(IDDM), group (3) included 21 children with schistosomal hepatic fibrosis (SHF) and group (4) included 20 children with chronic rheumatic heart disease (RHD). All were randomly selected from the Hepatology, Diabetology, Haematology and Cardiology outpatient clinics of Alexandria University Children's Hospital, Alexandria, Egypt. 110 healthy age matched children served as a control group. All the study children were subjected to thorough history taking, with special emphasis on previous episodes of jaundice, injections and blood transfusions. Informed consent for the blood testing was obtained from the parents of all children before including in the study. Full clinical examination was performed and the span of the liver recorded. Blood samples were collected for estimation of serum alanine transferase concentrations. All serum samples of the study children were tested for the presence of hepatitis B surface antigen (HBSAg) using the latex quick test (281) and IgG antibody to HCV by indirect ELISA test (Behring).

Analysis of variance(ANOVA) was used to test the differences between mean values in the study groups. Significance was accepted at $p<0.05$. Data are presented as mean +/- SD.

RESULTS: (see appendix for tables)

Table (1) presents the prevalence of HCV antibody, liver size and ALT concentrations in the five study groups. Children with SHF were significantly older than children in the other groups. The prevalence of HCV seropositivity was significantly higher in three of the high risk groups (thalassaemia, IDDM, and SHF) compared to the control group. In the RHD group the prevalence of HCV antibody was 0%. HBS antigenaemia was found in 7 of the normal children (6.4 per cent), 4 of the thalassaemic children (23.5 per cent), 2 of the diabetic children (11.8 per cent); 4 of the patients with SHF (19 per cent) and one patient with RHD (5 per cent). Liver span was significantly larger in the thalassaemic and SHF groups compared to the other groups. ALT concentrations were significantly higher in the thalassaemic and SHF groups versus the control group. In a large group of thalassaemic patients (r=72) ALT concentration was correlated significantly (negatively) with the HtSDS of all the study children (r= -0.42, p< 0.001) (figure 1).

Figure 1: Relation between ALT concentrations and HtSDS in 74 thalassaemic children (r= -0.42, p< 0.001).

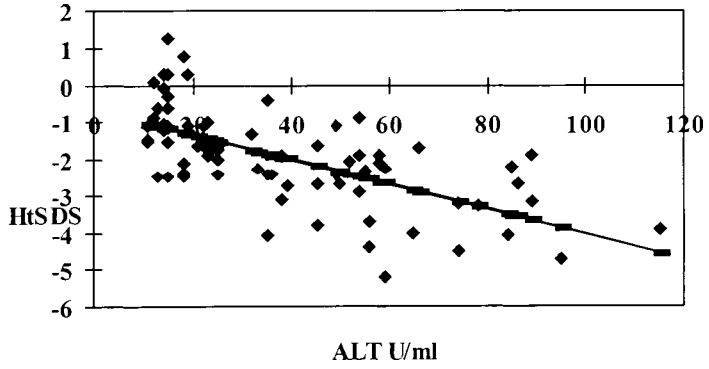


Table (2) compares ALT concentrations and liver span among the HCV antibody seropositive and seronegative children. In all the 5 study groups ALT concentrations were significantly higher in HCV seropositive compared to seronegative children. Liver span was significantly larger in HCV seropositive control children as well as those with thalassaemia and SHF Vs children who were seronegative in each group respectively.

DISCUSSION

Hepatitis-C virus can be detected in blood within 7-14 days of exposure and persists throughout the course of infection. However, the presence of circulating HCV

antibody can not be confirmed until 9-20 weeks after exposure. This creates a window period of seronegativity and potential infectivity (284,285). Acute infection carries a high risk of developing chronic hepatitis and liver cirrhosis. The HCV antibody test is used to detect acute and chronic HCV infections. It is positive in approximately 50 per cent of patients with acute HCV infection and 80-90 per cent with chronic NANB hepatitis (285,286,287).

In this study the prevalence of HCV antibody in normal (n=110) Egyptian children is 11.8 per cent. This is a markedly higher prevalence rate compared to those reported from different African and Asian countries (288-291). It appears that the risk and magnitude of HCV infection are relatively high in Egypt.

The major factors in community-acquired HCV infection include blood transfusions, intravenous drug abuse, and inapparent percutaneous exposure (286). One study has suggested that household and sexual transmission of HCV is most inefficient (292). In 3 of the high risk groups of children we studied , namely those with thalassaemia major, IDDM and SHF, the prevalence of HCV antibody positivity has been significantly high (44.4 , 29.3 and 38.1 per cent respectively). This confirms that parental transmission, either through intravenous or subcutaneous route, is a major factor in community-acquired infections. In addition, this high prevalence of HCV seropositivity and HBS antigenaemia in these groups of children poses a considerable danger for development of chronic liver disease. The presence of significantly larger liver span in HCV seropositive normal children as well as HCV seropositive children with thalassaemia and SHF and the significant elevation of their serum ALT concentrations compared to HCV seronegative children in each group respectively strongly support this view. Moreover, Masera and colleagues has reported high rate of occurrence of chronic liver disease following acute NANB-hepatitis in thalassaemic patients (218,219). The significant correlation between ALT concentrations and the HtSDS in children with thalassaemia ($n = 72$) ($r= -0.42$, $p<0.001$) suggested that impaired hepatic function might adversely affect linear growth in these children.

In conclusion, the prevalence of HCV antibody and HBSAg seropositivity is relatively high (11.8 and 6.4 per cent respectively) in a random sample (n=110) of healthy Egyptian children and significantly high in children with thalassaemia major (44.4 and 23.5 per cent respectively), and SHF (38.1 and 19 per cent respectively). These data denoted high prevalence of chronic hepatitis in these patients. Hepatic dysfunction

might contribute to the impaired linear growth in these children.

Study 4: CIRCULATING GROWTH HORMONE (GH), INSULIN-LIKE GROWTH FACTOR-I (IGF-I) AND FREE THYROXINE, GH RESPONSE TO CLONIDINE PROVOCATION AND CT SCANNING OF THE HYPOTHALAMIC-PITUITARY AREA AND RESPONSE TO GH THERAPY IN CHILDREN WITH SICKLE CELL

(Journal of Tropical Pediatrics 1995;41: 285-289),

Presented at the IIIrd European Congress of Endocrinology, Amsterdam, The Netherland July 1994. (Abstracted in the European J Endocrinology, 1994, 130, Suppl 2; 195.

AIM OF THE WORK:

1. to estimate the circulating levels of IGF-I, FT4, TSH and the responses of GH and cortisol to provocation by clonidine and adrenocorticotrophic hormone (ACTH) respectively in children with SCD with short stature (Ht below 5th percentile) for age and gender and analyse these hormonal data in relation to the anthropometric, clinical and biochemical data of the patients.
2. to study the anatomy of the hypothalamic-pituitary area , using CT-scanning, of short children with SCD who have abnormal GH/IGF-I axis.
3. to study the effect of GH therapy on linear growth in short children with SCD with defective GH/IGF-I axis.

PATIENTS AND METHODS:

Fifteen prepubertal children with SCD and short stature, HtSDS below -2, attending the Paediatric Haematology/Endocrinology Clinic of the Royal Hospital, Muscat, Oman, were the subjects of this study. All children were on regular blood transfusion to keep their Hb concentrations above 9g/dl. All were on folic-acid supplements and all had been vaccinated against pneumococci. Fifteen age-matched children with normal variant short stature (NVSS) served as controls. None of the children had history of intrauterine growth retardation, any other systemic or endocrine disease, dysmorphic trait or central nervous system irradiation. Informed consent for the testing procedure was obtained from the parents of all children. All the children were examined thoroughly with special emphasis on nutritional data. The anthropometric measurements included weight, Ht, BMI and MAC. The HtSDS and GV cm/yr for the

last year were calculated. Normal population data were according to the Tanner et al (277).The bone age was determined according to Greulich and Pyle atlas (278).
On the day of admission, venous blood samples were obtained for determination of complete blood count (CBC) and serum albumin, bilirubin, alanine transferase (ALT), alkaline phosphatase (ALP),calcium, phosphorus and bicarbonate concentrations. Following an overnight fast (8-h) venous blood samples were withdrawn through a polyethylene catheter inserted in a forearm vein between 8 and 9 a.m. The serum was separated from the formed elements by centrifugation and kept frozen at -20 c until analysed for GH, FT4, TSH and IGF-I. After obtaining the basal samples, an oral dose of clonidine was given (0.15mg/m2). Blood samples were obtained every 30 min for 2 hours for measurement of serum GH concentrations. On the next morning and after an 8-h overnight fast 0.5mg of ACTH (synacthen) was injected iv and blood samples obtained before and 60 min after the injection for estimation of cortisol concentrations. Human GH was measured by radioimmunometric assay.

CT examination of the hypothalamic pituitary area have been performed in all sicklers who did not mount an appropriate GH response to clonidine provocation (n=8). One of the children with SCD had a second CT performed after intrathecal injection of the contrast material.

Two short children with SCD, defective GH release , low IGF-I and empty sellae were treated for three years with human GH (15 units/m2/week, divided on daily subcutaneous doses given each night). Their linear growth was recorded for the three years.

Statistical analyses were done using the unpaired t-test to compare mean analyte concentrations among the 2 study groups when the data were normally distributed and Wilcoxon test when they were not. Statistical significance was accepted at $p<0.05$.

RESULTS

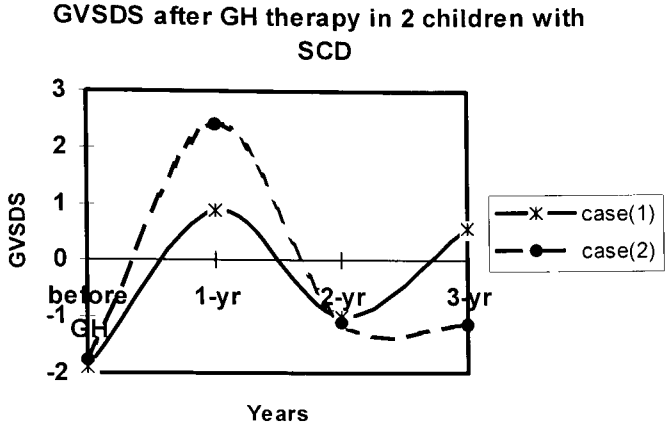
The chronological age, HtSDS, BMI and bone age did not differ significantly between the 2 groups (table 1). Creatinine clearance and serum concentrations of creatinine, bicarbonate, albumin and ALP were not different among the 2 groups (table 2). Hb concentrations and haematocrit values were lower in children with sickle cell disease

and their serum ALT and bilirubin concentrations were higher compared to the control children.

The circulating IGF-I concentrations and the peak GH levels in response to clonidine provocation were significantly lower in the sickle cell group (table 3). Eight out of the 15 children with sickle cell disease did not mount a proper GH peak >10ug/L after clonidine intake, whereas all children with NVSS mounted normal GH response >10ug/L. Basal and ACTH-stimulated cortisol levels as well as FT4 and TSH concentrations did not differ significantly between the 2 groups. CT scanning of the hypothalamic-pituitary area revealed a picture of complete (n=5) or partial (n=3) empty sella in all the 8 children with SCD who had defective GH response to clonidine. In those patients with complete empty sella the contents of the sella had densities around cerebrospinal fluid (CSF) values figure (1), whereas in those with partial empty sella the contents of the pituitary fossa were between -45 and -27 which was suggestive of some fatty degenerative changes figure (2). In the patient who had CT done after intrathecal injection of contrast medium figure (3) a significant herniation of the suprasellar cistern into the pituitary fossa was noted. Plain skull x-rays for the pituitary fossae were normal in all children with NVSS and SCD.

Human GH therapy (15units/m2/week, on daily divided subcutaneous doses) was started in two prepubertal children with SCD and ESS who had annual growth velocity (GV) of 3.2 and 3.5 cm/yr. respectively. Their GV increased to 9.7 and 8.1 cm/yr. on the first year of therapy and declined markedly to 5 and 4.1 cm/yr. and 3.6 and 3.9 cm/yr. on the second and third years of GH therapy respectively (figure 4).

Figure 4.



DISCUSSION

The hormonal profile of children with sickle cell disease revealed defective GH response to clonidine provocation as well as lower circulating concentrations of IGF-I compared to the control group. Malnutrition can be excluded as a cause of these changes because the BMI and serum albumin concentrations were not different among the 2 study groups. In addition, in malnutrition the basal GH concentrations are usually high unlike the case in our children with sickle cell disease (53,94). The normal creatinine clearance, and normal concentrations of serum bicarbonate, calcium, phosphorus and alkaline phosphatase exclude any contribution of renal dysfunction and/or disturbed calcium homeostasis in the aetiology of growth impairment or hormonal abnormalities in these children.

Although serum ALT and total bilirubin concentrations were higher in the group with sickle cell disease compared to the control group, those ALT levels of sicklers were still in the normal range of population (10-60 IU/L) and the unconjugated bilirubin constituted the main part of the elevated bilirubin. This in addition to normal concentrations of serum albumin and ALP and normal albumin/globulin ratio makes it unlikely that hepatocellular dysfunction is a cause for low IGF-I production in these patients.

In summary, it appears that in short children with sickle cell disease low GH secretion leads to decreased synthesis of IGF-I and consequently to slow growth velocity and short stature. In support of this view CT scanning of the pituitary region revealed an empty or partially empty sella in all the eight children with SCD who did not mount an appropriate GH response to clonidine stimulation. Defective GH release and empty sellae might be due to hypoxic injury or infarction to the pituitary gland during one or

more of the sickling episodes. In our child with severe SCD, and empty sella with cisternal herniation figure (3), there was marked reduction in his GH response to high dose clonidine (7ug/L) and to insulin-induced hypoglycaemia (6.5ug/L). This finding supports our hypothesis of hypoxic pituitary insults in these children. As far as we could ascertain, this observation has not been made before. Treatment of 2 children with SCD and GHD with human GH accelerated their linear growth during the first year of treatment with rapid deceleration during the next two years, suggesting only partial response to GH therapy in children with SCD. Further studies are required to clarify more the abnormality/abnormalities of the GH/IGF-I axis in these patients.

Study 5: GROWTH HORMONE (GH) SECRETION, AND CIRCULATING INSULIN-LIKE GROWTH FACTOR-I (IGF-I) AND IGF-BINDING PROTEIN-3 CONCENTRATIONS IN CHILDREN WITH SICKLE CELL DISEASE. *(Metabolism 1997,46: 1241-1245)*

(Presented at the Second Meeting of the European Haematology Association (May 29-June 1, 1996) Paris, France.
(Abstracted in the British J Haematology, 1996, 93, supplement 2, 34, Abst No 132.

AIM OF THE WORK:

To further test the hypotheses that :

1. SCD is associated with abnormalities of the GH/IGF-I/IGFBP axis; and

2. SCD is associated with GH resistance.

The first issue was addressed by measuring GH response to clonidine and glucagon, and the circulating concentration of IGF-I and IGFBP-3 in 21 children with SCD, and the second issue was tested by means of IGF-I generation tests. These results were compared with those for children with idiopathic short stature (ISS) (n=10) and isolated growth hormone deficiency (GHD) (n=11).

PATIENTS AND METHODS:

Twenty-one prepubertal children with SCD were the subjects of this study. They were randomly selected , using random tables, from 58 children with SCD and short stature (height 10th percentile for age and sex) . The 58 children were all the patients with SCD and stature <10th percentile selected from a cohort of 156 children with SCD regularly attending the Haematology outpatient clinic of the Royal Hospital, Muscat, Oman. According to their peak GH responses to 2 provocation tests (with clonidine and glucagon) they were divided into two subgroups. 9 children (group 1) had low peak GH response in two or more provocative tests (< 7ug/L) and the other 12 children (group 2) had normal GH response to stimulation (>7ug/L). All have been on folic acid supplement and vaccinated against pneumococci. None of them had history of intrauterine growth retardation, any other systemic or endocrine disease, dysmorphic trait or central nervous system irradiation. Ten age-matched children with idiopathic short stature (ISS) (HtSDS below -2, and normal GH response to provocation) and 11 children with isolated GHD (GH peak response < 5ug/L in two or more provocation

tests) served as controls. Informed consents were obtained from all the parents and when appropriate from children before including in the study.

All the children were examined thoroughly with special emphasis on the nutritional data . The auxological measurements included weight, height , head circumference, MAC, BMI and scapular, triceps and abdominal skin fold thickness. Normal population data were according to the Tanner et al (277). The bone age was determined according to Greulich and Pyle atlas (278).

Children were admitted to the Royal hospital, Muscat, Oman for the period of their investigation. Initial nutritional evaluation , both qualitative and quantitative, was performed by the dietician using the recall method and the patient received a high protein (2gm/kg/day) diet for 7 days before hormonal evaluation. After an overnight fast (8h) venous blood samples were obtained between 8 and 9 am for determination of complete blood count (CBC), serum albumin, bilirubin, alanine amino- transferase (ALT), bone-specific- alkaline phosphatase (ALP), calcium, phosphorus and bicarbonate concentrations. The serum was separated from the formed elements by centrifugation and kept frozen at -20 c until analysed for IGF-I, IGFBP3, cortisol, free thyroxin (fT4), and TSH by radioimmunoassay. The subjects received a single injection of 0.1 mg/kg recombinant human GH subcutaneously. Serum IGF-I and IGFBP-3 levels were remeasured 24 hours after the injection (293). Human GH and IGF-I were measured by radioimmunometric assays. IGFBP3 was measured by radioimmunoassay in Serono (SCL) Bioscience Services employing reagents supplied by Mediagnost.

Results are expressed as the mean +/- SD and analysed by ANOVA test to compare analyte concentrations among groups. Paired student-t test was used to compare data in the same group before and after GH injections. Correlation between variables of interest are examined by linear regression analysis and, when appropriate multiple regression analysis.

RESULTS: (see appendix for tables and figures)

Children with SCD and ISS had normal circulating concentrations of albumin, calcium, phosphate, ALP, ALT , bicarbonate. Serum levels of FT4, TSH and 8-A.M. cortisol did not differ among the two groups. Bilirubin (unconjugated) concentrations were higher

and haemoglobin (Hb) concentrations and haematocrit values were lower in the SCD group versus the ISS group. The peak D-xylose concentration after ingestion of 5 g D-xylose did not differ between the two groups .

Table I shows auxological and hormonal data of children with SCD, GHD and ISS. The HtSDS was significantly lower in children with ISS and GHD versus children with SCD. Linear growth velocity (GV) , GVSDS and BMI did not differ significantly among the two groups with ISS and SCD. However GV and GVSDS were significantly decreased in children with GHD. The peak GH response to provocation was significantly lower in children with SCD Vs ISS ($p<0.001$). Basal circulating IGF-I concentrations were higher in children with ISS Vs the other two groups. Basal circulating IGFBP3 concentrations were significantly lower in the SCD group ($p<0.05$) Vs those for ISS group. The IGF-I response to GH administration (IGF-D; equal to the 24 h IGF-I value minus the basal value) was significantly lower in children with SCD (27.6 +/- 15.6 ng/mL) compared with those for children with ISS (53.5 +/- 17.6 ng/mL). The concentrations of IGF-I increased significantly after human GH injection in the three study groups. However, the IGF-I response (IGF-I-D) was significantly higher in children with GHD and those with ISS Vs those with SCD.

Table II presents a comparison between two groups of children with SCD : Group-1 with defective GH secretion (n=9) (peak GH response to provocation = 4.2 +/- 2.3 ug/L) and group-2 (n=12) with normal peak GH response to provocation = 9.9 +/- 2.4 ug/L. The age and BMI did not differ among the two groups. The HtSDS and GVSDS were significantly lower in group-1. The basal circulating concentrations of IGF-I and IGFBP3 were significantly lower in group I (SCD with defective GH secretion). The IGF-I and IGFBP3 responses to GH administration did not differ significantly among the two groups and were significantly lower compared to those for children with isolated GHD.

Correlations between auxological and hormonal data in the 21 children with SCD are presented in Table III. The basal and GH-stimulated concentrations of IGF-I and IGFBP3 were correlated significantly with height GV and GVSDS ($p<0.01$) on the one hand and GH peak values on the other hand. The dependence of serum IGFBP3 on GH was evident from a significant correlation with peak GH secretion ($n=21$, $r= 0.665$, $p<0.01$) and the significant increase of IGFBP3 concentrations after one injection of GH. IGFBP3 concentrations correlated significantly with IGF-I levels before and after

GH administration (r= 0.600, p< 0.01). Serum ferritin concentration was inversely correlated with GV, GVSDS, and IGF-I (r= -0.35, -0.46, and -0.25 respectively, P<0.05)

DISCUSSION

There is no completely reliable test for diagnosing or excluding GH deficiency in all short children. When used alone, none of the tests has superior diagnostic specificity or sensitivity. Measurement of GH-responsive peptides, such as IGF-I and IGFBP-3, can add insights even when results do not agree with GH responses to provocative stimuli. Interpretation of the tests together improves the reliability of diagnostic assessment (158).

Nine out of the 21 (43%) randomly selected children with SCD had defective GH secretion in response to provocation with both clonidine and glucagon. In these children the IGF-I concentrations were comparable to those for children with isolated GHD, but significantly lower than those for children with ISS and normal GH release. Their circulating IGFBP3 concentrations, which reflect the integrated GH secretion over days (158,294,295), were significantly lower versus the group with normal GH secretion, but not different from those reported by Smith et al. (158) for children with GHD. The linear growth of patients with SCD with defective GH secretion was significantly slower than those with normal GH secretion. This defective GH secretion might be secondary to a hypoxic-vascular insult to the hypothalamic pituitary area during one or more of the sickling episodes and/or pituitary atrophy as a result of iron overload. In support of this view CT scanning of the pituitary region revealed an empty sella (partial, or complete) in all the 9 children with SCD and GHD. Moreover, the SCD severity score , as described by ElHazmi et al (260) was significantly higher in the SCD group with defective GH secretion versus those with normal GH secretion . Collectively these findings suggest that acquired GH deficiency may be a major factor in the aetiology of retarded growth in some patients with SCD especially those with severe sickling attacks.

It is unclear which of the alterations of GH/IGF-I/IGFBP axis that are found in children with SCD are of more clinical significance. Although the influence of SCD on adult height is controversial, most data indicate a negative effect of SCD on linear growth (1-4,6). In our study, mean HtSDS for all the studied patients with SCD (n=21)

(-1.34 +/- 0.6) was in the lower range of normal . Those SCD patients with defective GH secretion had significantly lower HtSDS and GV compared to those with normal GH release. Moreover, we found a good correlation between GVSDS and IGF-I (before and after GH injection) on the one hand and a strong correlation between peak GH response to provocation and IGF-I and IGFBP3 on the other hand. These findings suggest that circulating IGF-I and IGFBP3, as in normal children, are the major determinant of linear growth in children with SCD, both are regulated by the GH status of the child. On the other hand the degree of GH resistance , as measured by the change of IGF-I concentration after GH injection (IGF-I-D) was not correlated with linear growth parameters (HtSDS, GV, GVSDS) (r= -0.26, -0.002 and 0.083 respectively) in these children. This might denote that GH deficiency plays a more significant role than GH resistance in the aetiology of impaired linear growth in these children.

In children with SCD, growth impairment includes both height and weight (199-203). Treatment with human GH has been shown to improve nitrogen balance and linear growth in a variety of growth disorders (296) . The detection of defective GH secretion in about 40 per cent of slowly growing children with SCD suggests a beneficial growth promoting effect of GH therapy in these patients. The IGF-I generation test has been used to assess IGF-I responsiveness to GH in children with short stature(36). In this study, a single, subcutaneous injection of GH has been used to investigate the physiological changes of IGF-I/IGFBP3 in children with SCD to test the hypothesis that SCD is associated with GH resistance. Subjects with SCD , with or without defective GH secretion, had significantly lower IGF-I responses to a single injection of GH compared to children with ISS and those with isolated GH deficiency, suggesting partial GH resistance. This might attenuate the anabolic effects of GH therapy in these children (297). The IGF-I response (IGF-I-D) correlated negatively with BMI (r= -0.31, P<0.01) suggesting that in children with SCD increasing wasting may be associated with progressive GH resistance. The significant negative correlation between IGF-I response to GH injection and peak GH response to provocation (r= -0.505, P<0.01) suggests that IGF-I production is better in those children with defective GH secretion. However, the IGF-I response of children with SCD and GHD was insignificantly higher versus those with normal GH secretion.

Malnutrition can be excluded as an important cause of abnormalities of the GH/IGF-I axis in children with SCD because of the following: 1. the BMI , subcutaneous fat thickness and mid-arm circumferences and serum albumin concentrations were not

different among the study groups (SCD, SCD +GHD, and ISS); 2. analysis of their nutritional history proved adequate , qualitative and quantitative, intake of nutrients compared with normal 20-age matched children; 3. D-Xylose test was normal in all the children with SCD and those with ISS; 4. in malnutrition the basal GH concentrations are usually high unlike the case in our children with SCD (mean +/- SD= 1.8 +/- 0.25 ug/L, range : 0.5-3.5 ug/L) (43).

The normal serum creatinine , bicarbonate, calcium, phosphorus, and ALP exclude any significant contribution of renal dysfunction, and/or disturbed calcium homeostasis in the aetiology of growth impairment or endocrine abnormalities in these children. Normal serum concentrations of albumin, ALP, and alanine amino-transferase (ALT) concentrations with normal albumin/globulin ratio and prothrombin time in children with SCD makes it unlikely that impaired hepatocellular synthetic function is a cause for low IGF-I production in these patients.

Serum ferritin concentration, as an indicator of body iron stores, was negatively correlated with height GV ($r= -0.35$, $P<0.01$), GVSDS ($r=-0.464$, $P<0.01$), and circulating concentrations of IGF-I ($r=-0.255$, $P<0.05$). This suggests that hepatic/parenchymal iron overload may impair IGF-I synthesis and subsequently slow linear growth in children with SCD.

In summary, children with SCD have significantly lower IGF-I production in response to GH injection compared to those with ISS and GHD suggesting partial GH resistance. Some children with SCD and delayed growth may have GH deficiency. Parenchymal iron overload, a potentially treatable factor, may contribute in the aetiology of impaired hepatic IGF-I synthesis and/or defective GH secretion by the pituitary gland. Because of the presence of partial GH resistance treatment with recombinant IGF-I might be more successful than treatment with GH.

Study 6: EMPTY SELLA IN SHORT CHILDREN WITH AND WITHOUT HYPOTHALAMIC-PITUITARY ABNORMALITIES, INCLUDING CHILDREN WITH SICKLE CELL DISEASE.

(Indian Journal of Pediatrics 1995;62:75-81),

(Abstracted in The European Journal of Endocrinology 1994; 130; Suppl 2, 144)

(Presented at The XXI International Congress of Pediatrics, Cairo, Egypt, September,1995).

AIM OF THE WORK:

The finding of high incidence of empty sellae in short children with sickle cell disease has stimulated us to conduct this comparative study to investigate the growth parameters, static and dynamic pituitary function testing and CT imaging of the hypothalamic-pituitary area in short children with SCD and compare these data with those for two other groups of short children with and without hypothalamic-pituitary abnormality. These two control groups included children with GH deficiency and those with normal variant short stature (NVSS) respectively .

PATIENTS AND METHODS

Ninety prepubertal (Tanner stage 1) children with age range between 4 and 10 years were the subjects of this study. They included: A) 15 children with SCD and GH deficiency, they were diagnosed out of 34 children with SCD and growth retardation presented to the same clinic during the same period, B) 25 children with GH deficiency , 5 with multiple pituitary deficiency including GH,TSH and ACTH , and 20 with isolated GH deficiency, they were all the diagnosed cases in the Endocrine clinic of the Royal Hospital, Muscat, Oman between April 1991 and August 1993, C) 30 children with NVSS (Height = 2SD or more below the mean for age and gender) who were randomly selected from all the children with NVSS who attended the Endocrinology clinic and investigated between January 1992 and January 1993 (30 out of 95), and D) 20 age matched children with normal stature (HtSDS between -1 and +1), they were selected from the radiology department while taking CT scans for other reasons (15 with head trauma to role out intracranial lesions and 5 to role out intraocular pathology). All of them had no pathology in their CT. All the children with SCD were

on regular blood transfusion to keep their haemoglobin (Hb) concentrations above 9g/dl and folic acid supplements. None of the children in this study had history of intrauterine growth retardation, malnutrition, any other systemic disease, dysmorphic trait, encephalitis ,intracranial lesions or central nervous system irradiation. Informed consent for the testing procedure and CT scanning was obtained from the parents of all children. CT scans of the brain with 1mm cuts in the hypothalamic pituitary area were evaluated by our Neuroradiologist (AD). Empty sella was defined as sella that, regardless of its size, is completely or partly filled with CSF (5,6). The anthropometric measurements including weight (Wt) and height (Ht) were recorded using the Harpenden's anthropometric measuring instruments . The data recorded were the average of three sequential measurements determined by the same observer (AST). The HtSDS, height GV cm/yr. and GVSDS were calculated. Normal population data were according to Tanner et al. (277) The body mass indices (BMI) were calculated according to the formula BMI= Wt (kg)/Ht (m)2. The bone age was determined according to Greulich and Pyle atlas (278).

On the day of admission, venous blood samples were obtained for determination of complete blood count (CBC) and serum albumin, bilirubin, alanine transferase (ALT), alkaline phosphatase (ALP), calcium, phosphorus and bicarbonate concentrations. Following an overnight fast (8-h) venous blood samples were withdrawn through a polyethylene catheter inserted in a forearm vein between 8 and 9 a.m. The serum was kept frozen at -20 c until analysed for GH, free T4 (FT4), TSH, and IGF-I. After obtaining the basal samples, an oral dose of clonidine (0.15mg/m2) was given and venous blood collected every 30 minutes for 2 hours for measurement of serum GH concentrations. On the next morning and after an 8-h overnight fast 0.1 mg of glucagon (Maximum of 1mg) was injected i.m. and blood samples obtained before and every 30 min after the injection for 180 min for estimation of GH and cortisol concentrations (9). The first urine sample passed in the morning was analysed including specific gravity and osmolality. On the third day and after an overnight fast oral glucose 1.75gm/kg (20% solution) was given to the child and blood collected before and 120 minutes after the oral glucose load for determination of plasma glucose concentration by glucose oxidase method. Human GH and IGF-I, FT4, TSH and cortisol concentrations were measured by radioimmunometric assays.

RESULTS: (see appendix for tables)

GV was significantly slower and GVSDS was lower in children with GH deficiency Vs those with SCD+GH deficiency(table 1). The bone age was significantly delayed in the 3 groups of children with short stature compared to the controls, however the degree of delay did not differ among the 3 groups.

The biochemical and haematological data of the 4 study groups are presented in table (2). Creatinine clearance , and serum concentrations of albumin, ALT, ALP, bicarbonate, calcium, phosphate and albumin did not differ among the 4 study groups. None of the patients had urine specific gravity below 1015 or osmolality below 465 mmol/kg ruling out diabetes insipidus.

Table (3) shows the hormonal profile and glucose data of the 4 study groups. Out of the 25 children with GH deficiency, 20 (80 per cent) had isolated GH deficiency, and 5 (20 per cent) had multiple pituitary deficiency (GH , TSH and/or ACTH). All the growth retarded children in the sickle cell group, who had defective GH response to stimulation (GH peak<10 ug/L), had empty sella (100 per cent) , and their FT4,TSH , 8AM cortisol concentrations and cortisol response to glucagon were normal. The IGF-I concentrations were significantly lower in the 2 groups of children with GH deficiency compared to those with NVSS and normal children. Empty sella was noted in 9 out of the 20 children with isolated GH deficiency (45 per cent), 4 out the 5 children (80 per cent) with multiple hypothalamic-pituitary hormonal deficiencies (none with diabetes insipidus), all the children with SCD associated with GH deficiency, 3 out of 30 children with NVSS (10 per cent) and in none of the normal children. Children with isolated GH deficiency and ESS had normal BMI for age and gender (15.4 +/- 1.7), whereas children with SCD and ESS had BMI (11.1+/-0.7) below the 5th percentile for corresponding age and gender. None of the children had glucose intolerance or high blood pressure.

DISCUSSION.

In this study the incidence of empty sella was 45 per cent in children with isolated GH deficiency , 80 per cent in those with multiple pituitary deficiency, 100 per cent in children with SCD and GH deficiency and 10 per cent in the short children with normal hypothalamic-pituitary function. None of the children with normal stature had empty sella. These findings suggest that empty sella has a considerably high incidence in

children with growth and hypothalamic pituitary disorders, particularly those with multiple pituitary hormone deficiency . This implicates the importance of CT scanning of the hypothalamic-pituitary area, in children with severe short stature, as an important tool in the identification of children with pituitary hormonal deficiency. Our findings are in agreement with the study of Surtees et al (298) who reported empty sella in 90% of patients with multiple pituitary deficiency and in 37 per cent of patients with isolated GH deficiency. However, Marwaha et al (299) reported empty sella in 16 out of 22 children affected by isolated GH deficiency born by normal delivery (72.7 per cent), such difference may be partly due to their use of MRI which is more sensitive in the diagnosis of empty sella . In addition, some of their patients (23 per cent) had brain anomalies in the scan, a characteristic which was not found in our patients.

The association of empty sella and diabetes insipidus has been reported previously in CT scans (300,301), however none of our patients with primary empty sella nor those with empty sella and SCD had diabetes insipidus. This finding is in agreement with those of Cacciaricet et al (301) who reported absence of diabetes insipidus in their patients with primary empty sella without other anatomical abnormalities (n=23). It seems that the presence of intrasellar CSF does not have an adverse effect on posterior lobe function.

The presence of empty sella in all the sickle cell children who did not mount normal GH response to pharmacological stimuli suggests that ischaemic injury to the pituitary during the sickling episodes might be the cause of both GH deficiency and empty sella in these children.

None of the children with empty sella had symptoms or signs of increased intracranial pressure, obesity or glucose intolerance reported in adults with empty sella (302). In this study the significantly low BMI in children with SCD + empty sella and normal BMI in children with isolated GH deficiency and empty sella exclude an important effect, if any, of empty sella on the control of body weight in children.

In summary, empty sella is frequently seen in children with GH deficiency secondary to SCD and those with idiopathic GH deficiency but it is not rare in very short prepubertal children with normal hypothalamic-pituitary function. In short patients with SCD; the finding of empty sella was associated with isolated GH deficiency, with

normal thyroid, adrenal, and posterior pituitary functions. Empty sella is not associated with impaired glucose tolerance or obesity in these children.

Study 7: INSULIN AND GLUCAGON RESPONSES TO PROVOCATION WITH GLUCOSE AND ARGININE IN PREPUBERTAL CHILDREN WITH THALASSAEMIA MAJOR BEFORE AND AFTER LONG-TERM BLOOD TRANSFUSION.

(Journal of Tropical Pediatrics 1996;42:291-296.)

Presented at XIIIth meeting of The International Society of Haematology, Istanbul, Turkey, September 3-8 1995

(Abstracted in Pediatrics 1995; 95:4, supplement 2: 9-10)

AIM OF THE WORK:

1. To evaluate prospectively insulin and glucagon secretion, in response to oral glucose and arginine infusion, in young thalassaemic children before and after long term high-transfusion and iron chelation; and
2. to evaluate GH and cortisol responses to insulin-induced hypoglycaemia and serum concentrations of IGF-I, free thyroxin (FT4) and TSH in thalassaemic children after long-term high-transfusion.

SUBJECTS AND METHODS.

Twenty young children with thalassaemia major , diagnosed between January 1988 and January 1990, were selected from the Haematology clinic of Alexandria University Children's Hospital for the study. Their age ranged between 1.75 and 3.5 years (mean =2.8 +/- 0.6 years). None had family history of diabetes mellitus, intrauterine growth retardation or any other systemic or endocrine disease. Informed consent was obtained from all the parents of the children before including in the study and the Ethical Committee of Alexandria University approved the study protocol. Before receiving the first blood transfusion children were admitted and offered high carbohydrate diet for 3 days. On the 4th day and after an 8-h overnight fast a standard oral glucose tolerance test was performed and serum collected at 0, 60 and 120 minutes after oral glucose load (1.75g/kg) for estimation of glucose concentration by glucose oxidase method and glucagon and insulin concentrations by radioimmunoassay using kits purchased from Amersham. On the 5th day and after an overnight fast an i.v. infusion of 10% arginine HCl infusion (0.5g/kg) was started and continued for 30 minutes and serum samples

obtained before and at 30 and 60 minutes after the start of the infusion for estimation of glucose, insulin and glucagon concentrations.

Children were started on frequent transfusion therapy (15ml of packed erythrocytes/kg of body weight given every 4 weeks to maintain the haemoglobin level at 9-10g/dl), and deferoxamine (50mg/kg/dose for up to six days per week) given by i.m. injection. During each clinic visit the children were examined thoroughly with emphasis on nutritional and growth data and their anthropometric measurements including weight and height recorded. Harpenden's callipers and anthropometric measurements were used. The HtSDS, GV (cm/yr) were calculated for each year and recorded. Normal population data were according to Tanner et al (277). Lab investigations included complete blood count and estimation of serum concentration of albumin, bilirubin, alanine transferase, urea and creatinine at 6 month intervals.

After a period of 2.5 to 4 years (mean = 3.1 +/-0.6 years) of high-transfusion and chelation 15 out of the 20 patients were admitted and the previous hormonal investigations redone. Three children were excluded from the study because of poor compliance and the other two because parents refused to give consent for re-testing. In addition, after an overnight fast a basal serum sample was collected for measurement of GH, cortisol, IGF-I, FT4, and TSH concentrations. GH response to oral clonidine (0.15 mg/m2) was evaluated in all the thalassaemic children and serum collected at 0,60 and 90 minutes after clonidine intake for estimation of GH . 15 normal age-matched children served as controls for the hormonal and biochemical studies after taking parents' consents. Human GH, IGF-I FT4, TSH and cortisol were measured by radioimmunometric assay. Skeletal age was determined according to the Atlas of Greulich and Pyle (278).

Statistical analyses were done using the ANOVA test to compare analyte concentrations before versus after long-term blood transfusion when the data were normally distributed and Wilcoxon test when they were not. Statistical significance was accepted at $p<0.05$.

RESULTS: (see appendix for tables)

Glucose, glucagon and insulin data are presented in tables 1 and 2. During fasting, serum insulin and glucose concentrations did not differ among the study groups. Serum

glucagon was significantly higher in thalassaemic children after Vs before frequent blood transfusion. After oral glucose load insulin secretion was significantly reduced in thalassaemic children after Vs before long-term high-transfusion. Their serum insulin/glucose (I/G) ratios were significantly lower {(0.27+/-0.12) at 1-h and (0.28+/-0.13) at 2-h} Vs before therapy {(0.62+/-0.26) at 1-h and (0.55+/-22) at 2-h }. In thalassaemic children on long-term blood transfusion serum glucagon was not suppressed after an oral glucose load. Serum glucagon levels were significantly higher at 60 and 120 minutes after the oral glucose load compared to values before therapy and to those for the control children. None of the thalassaemic children had impaired glucose tolerance after this period of frequent blood transfusion and chelation, however their serum glucose levels were significantly higher, at 60 and 120 min after the oral glucose load, compared to those for the control group. Thirty minutes after starting arginine infusion serum insulin concentration was significantly lower in thalassaemic children after long-term high-transfusion Vs before therapy. Basal and arginine-stimulated glucagon concentrations were significantly higher in thalassaemic children on frequent blood transfusion Vs before therapy. Consequently, serum insulin/glucagon ratios at 0, 30 and 60 min after starting arginine infusion were significantly decreased in thalassaemic children after (0.10+/-0.06, 0.08+/-0.05 and 0.11+/-0.07) versus before therapy (0.15+/-0.07, 0.17+/-0.08, and 0.15+/-0.07).

Table (3) presents growth parameters and hormonal data of thalassaemic children after a mean transfusion period of 3.1+/-0.6 years. The HtSDS of thalassaemic children decreased significantly from -0.75+/-0.3 before starting blood transfusion to -1.65 +/- 0.5 at the time of re-testing. HtSDS, GV and BMI were significantly lower in thalassaemic children compared to controls. Serum ferritin, bilirubin and ALT concentrations were significantly higher in thalassaemics Vs controls. Two out of the thalassaemic children were hepatitis-B surface antigen positive but none of the controls was positive. In all the thalassaemic children serum glucose concentration dropped significantly to <50% of the fasting level after injecting insulin (0.1 U/kg) intravenously. Thalassaemic children had significantly reduced peak GH response to both arginine and clonidine, with significantly lower serum concentration of IGF-I. Basal (8-h) cortisol , FT4 and TSH concentrations did not differ among the two groups. Serum ferritin concentrations were correlated significantly (negatively) with peak insulin and glucagon responses to arginine ($r = -0.48$ and $r = -0.42$ respectively, $p<0.01$) and with IGF-I concentrations ($r = -0.51$, $P<0.01$) in all the study children.

DISCUSSION

Although the basal serum insulin concentration was normal in thalassaemic children after long-term blood transfusion, there was a significant decrease in the maximal insulin secretory capability in response to arginine infusion and oral glucose load indicating a reduced insulin reserve. This gradual and early decrease of insulin secretion in thalassaemic children, before the occurrence of impaired glucose tolerance, proves that decreased B-cell mass plays a major role in the later development of glucose intolerance.

In patients with chronic pancreatitis and pancreatic fibrosis the fasting plasma glucose concentration remains normal as long as 20-40 per cent of the B-cell mass is retained. Nonetheless, loss of 20-40 per cent of B-cell mass is associated with a marked impairment in glucose-mediated insulin release, whereas a normal response to arginine is still retained. A further reduction (by 40-60 per cent) in B-cell mass leads to an altered response to arginine. Finally, when the B-cell mass is reduced by more than 80-90 per cell, fasting hyperglycaemia and inability to secrete insulin in response to all secretagogues is present (303, 304). It appears that in thalassaemic children on frequent and prolonged blood transfusion early iron deposition and gradual fibrosis of the pancreas (222) leads to gradual loss of B-cell mass and impaired response to secretagogues, similar to that of chronic pancreatitis. In addition, impaired insulin secretion in response to both oral glucose and arginine denotes a significant loss of B-cell mass (40-60 per cent) after a mean of 3.1 years of frequent blood transfusion in these patients. Our results support the findings of Dmochowski et al (236) indicating a trend towards progressive reduction in circulating insulin levels in thalassaemic patients

Although the presence of insulin resistance in patients with thalassaemia has been suggested by numerous studies (229, 230,235), the finding is not universal. Brianda et al (305) performed euglycaemic insulin clamps in thalassaemic patients and found increased insulin sensitivity. Our data showed that Despite hyperglucagonaemia serum glucose concentrations decreased properly (< 50 per cent of the fasting glucose level) after an intravenous insulin (0.1 U/kg) injection in all the thalassaemic children ruling out the presence of a significant insulin insensitivity at this age. However, prolonged hyperglucagonaemia and progressive hepatic dysfunction might add an element of insulin resistance at an older age.

In thalassaemic children on long-term transfusion the elevated basal serum glucagon concentrations, the significantly higher levels of glucagon and lower insulin/glucagon ratios after provocation with arginine in comparison with controls, and the non suppression of circulating glucagon levels after oral glucose load indicate a state of hyperglucagonaemia in these children. In other diseases with progressive fibrosis of the pancreas, inflammation and destruction of the parenchyma is associated with collapse between islets leading to formation of clusters of pancreatic islets. Within the remaining islets, a rearrangement of the endocrine cell population occurs with a proportionally greater loss of B-cells compared with A-cells, leading to a reversal of the normal 2:1 ratio (306). This quantitative change of the islet cells might explain in part the finding of hyperglucagonaemia in our children with thalassaemia. In addition, the A-cells are believed to be an insulin-dependent tissue, since inhibition of glucagon secretion by glucose normally occurs in the presence of insulin (307). In our thalassaemic patients impaired insulin secretion in response to glucose load might have contributed to the non-suppression of hyperglucagonaemia . The subnormal GH and cortisol peak responses to provocation exclude any contribution of these anti-insulin hormones in the production of insulin-resistance .

The progressive growth impairment in our thalassaemic children might be attributed to their low GH secretion and consequently low generation of IGF-I. These data support the theory that functional damage in hypothalamic structures for GH control is an important factor contributing to growth delay in these children (34-36). Although hepatic GH receptor and/or postreceptor defect might explain the low IGF-I generation in thalassaemic children with normal GH secretion (255), Postel et al (307), did not find evidence for a defect in GH binding to liver membranes in thalassaemic patients and there was no correlation between the level of GH binding to liver membranes and the degree of hepatic siderosis and fibrosis . Moreover, insulin plays an important role in determining the bioavailability of IGF-I through its action on insulin-like growth factor binding protein-1 (IGFBP-1) (89,90). Therefore, defective insulin secretion in thalassaemic children on long-term blood transfusion might increase hepatic production of IGFBP-1 leading to decreased bioavailability of IGF-I.

In summary, thalassaemic children on long-term high-transfusion and iron chelation develop progressive and early loss of B-cell mass manifested by decreased insulin release in response to secretagogues before impairment of their glucose tolerance.

However, hyperglucagonaemia and progressive hepatic dysfunction might add an element of insulin resistance later in life. The low BMI and slow linear growth in thalassaemic children might be explained in part by the defective secretion of the 2 major anabolic hormones, namely insulin and IGF-I. However, further studies are required to investigate the different factors affecting IGF-I secretion/action in these patients for better understanding of the aetiology of their impaired growth.

Study-8: INTERLEUKIN-1-BETA, TUMOUR NECROSIS FACTOR-ALPHA, ISLET CELL ANTIBODY, AND INSULIN SECRETION IN CHILDREN WITH THALASSAEMIA MAJOR ON LONG-TERM BLOOD TRANSFUSION. *(Journal of Tropical Pediatrics 1996;42: 362-364.)*

AIM OF THE WORK:

Recent studies reported increased production of different cytokines in multitransfused thalassaemic patients (308,309). These cytokines might have a role in the pathogenesis of diabetes in thalassaemic patients through their cytotoxic effect on islet cells and/or inhibition of insulin release (310-212).

To clarify this issue we measured the circulating levels of IL-1-B, TNF-A and islet cell antibody (ICA) in 20 children with IDDM, 20 of their non-diabetic siblings , 20 children with B-thalassaemia major and 10 normal age-matched children. In addition, we investigated the first phase insulin release and oral glucose tolerance (OGT) in the non-diabetic and thalassaemic children.

PATIENTS AND METHODS.

Twenty children with IDDM, 20 non diabetic children randomly selected from their siblings, and 20 children with B-thalassaemia major were the subjects of this study. 10 healthy children (mean age 7.8 +/- 1.6 years) with no family history of diabetes served as controls. Informed consents were obtained from the parents of all children and when appropriate from the children and The Ethical Committee of the University of Alexandria approved the study. Children were admitted to Alexandria University Children's Hospital for the study. All the children were prepubertal, clinically free from infection and had no other systemic diseases. None of them had history of recent viral infection. All the control children had normal hepatic and renal functions and haemogram. Thalassaemic children were on frequent transfusion therapy (15ml of packed erythrocytes / kg of body weight given every four weeks) to keep their haemoglobin at or above 10 g/dl and iron chelation therapy using i.m. deferoxamine. All had normal thyroid function and none of them had family history of diabetes. None of the diabetics had pancreatic calcification in either plain abdominal x-ray films or by sonographic examination.

A venous blood sample was obtained from all the children for measuring islet cell antibody (ICA), by the indirect immunofluorescent method (INOVA Diagnostics), and IL-1-B and TNF-A using ELISA technique. After 3 days on full carbohydrate diet an oral glucose tolerance test was performed (1.75 g glucose/kg of body wt) and serum glucose measured by glucose oxidase method before and 1 and 2 hours after the oral glucose load for all the non-diabetic children. On the second morning and after an overnight fast a glucose load (0.3 g/kg, 30 per cent solution) was injected iv. over 2 minutes and blood collected before and 5 minutes after the injection for measuring insulin concentration by radioimmunoassay.

Statistical analyses were done using the ANOVA test to compare mean analyte concentrations among the study groups when the data were normally distributed and Wilcoxon test when they were not. Statistical significance was accepted at $p< 0.05$.

RESULTS: (see appendix for tables)

IL-1-B and TNF-A concentrations were significantly higher in the IDDM-siblings group Vs those for the other groups (table 1). IL-1-B and TNF-A concentrations did not differ significantly among the thalassaemic group, diabetic group and healthy controls. Five of the thalassaemic children had impaired glucose tolerance after oral glucose load (25 per cent). All the IDDM-siblings had normal oral glucose tolerance. The early phase of insulin release was significantly lower in thalassaemic children compared to normal children and IDDM-siblings. ICA were detected in 30 per cent of the diabetic children(group II) Vs 60 per cent of their siblings. None of the thalassaemic children had detectable circulating ICA.

DISCUSSION.

Cytokines, through modulation of T-cell responses, can induce round cell infiltration (insulinitis) (313) and inhibit insulin release by the B-cells (311,312). In the IDDM-siblings the significantly high serum levels of IL-1-B and TNF-A, in conjunction with high prevalence of ICA suggested that these cytokines play important role in the autoimmune aggression against the islet B-cells. Despite their normal OGTT, the per cent increment of their insulin levels after iv. glucose was significantly lower than the control group. Campbell IL et al (313) suggested that B-cell destruction, in genetically susceptible subject, progresses through stages. Stage II; commences with infiltration of islets by immunoinflammatory cells (insulinitis), and production of cytokines from infiltrating cells. This induces "phenotypic switching" of islet cells. Stage III;

encompasses autoimmune-mediated destruction of B-cells by the targeted delivery of cytotoxic cytokines and other mediators. The normal serum levels of cytokines in children with IDDM can be explained by the fact that cytokine production is suppressed after extensive destruction of the target cells (B-cells).

In thalassaemic children on frequent blood transfusion, the initiation of immune reaction against the B-cells, by the excessive iron deposition and/or by the foreign cellular elements in the transfused blood , constitutes a possible pathogenic factor. However, the normal serum concentrations of IL-1-B and TNF-A and the absence of circulating ICA in thalassaemic children despite their high prevalence of impaired glucose tolerance and decreased insulin response to iv. glucose, exclude an important role, if any, played by the immune system in the aetiology of diabetes in these children.

In summary, it appears that over time children with thalassaemia experience a reduction in their circulating insulin levels which leads to glucose intolerance and diabetes mellitus. This B-cell destruction is mediated neither by ICA nor by cytokine production.

Study-9: GROWTH HORMONE (GH) RESPONSE TO PROVOCATION, CIRCULATING INSULIN-LIKE GROWTH FACTOR-I (IGF-I) AND IGF-BINDING PROTEIN-3 CONCENTRATIONS, IGF-I GENERATION TEST AND CLINICAL RESPONSE TO GH THERAPY IN CHILDREN WITH BETA-THALASSEMIA.

European J Endocrinology 1998; 138; 394-400.
Presented at the 79th Annual Meeting of The Endocrine Society, Minneapolis, June 11-14, 1997, USA.

AIM OF THE WORK:

1. To investigate the GH/IGF-I/IGFBP3 axis in prepubertal children with beta thalassaemia major,
2. to test the hypothesis that these patients might have GH resistance and;
3. to study the effect of GH therapy for one year on their growth parameters.

PATIENTS AND METHODS

Fifteen prepubertal children, between the age of 7.5 - 14 years, with B-thalassaemia major were randomly recruited for the study from the thalassaemia clinic of the Alexandria University Children's Hospital, Alexandria Egypt for the study. All have been treated with a chronic low transfusion regimen (to keep their haemoglobin above 8g/dl) , with IM chelation treatment (3 times/ week) (suboptimal chelation due to economic reasons) and were taking 5 mg of folic acid per day. Fifteen prepubertal age-matched children with constitutional short stature (CSS), age range 7- 13 years, (with height standard deviation scores at or below -2 and normal GH response to provocation) and 11 children with isolated GH deficiency (GHD) , age range 6.5 - 10.5 years, (GH peak response <5 ug/L in two or more provocative tests) served as controls. Informed consent was obtained from the parents of all the patients and when appropriate from the children before including in the study. The ethical Committee of Alexandria University has approved the protocol of the study. None of the children had intrauterine growth retardation, severe malnutrition, diabetes, dysmorphic trait, exposure to irradiation, or any other systemic illness. All patients were prepubertal, with pubarche Tanner 2 or less, gonadarche stage in the boys of 1 and thelarche stage in the girls of 1 and all were euthyroid

Anthropometric measurements included weight, height, MAC, and triceps-skinfold thickness. Growth velocity cm/yr. was measured over a whole year for all the patients before starting any GH therapy. HtSDS and BMI were calculated. Normal population data were according to Tanner et al . (277) Nutritional assessment included evaluation of dietary intake using the recall method for the previous 3 days . These data were recorded every clinic visit for the whole year (minimum of 3 visits/year).

After an overnight fast a venous sample was withdrawn for measurement of free thyroxin (FT4) , thyrotropin (TSH), insulin-like growth factor-I, IGFBP3 , and 8 A.M. cortisol concentrations. Serum ferritin, albumin, globulin, bilirubin, alanine transferase (ALT), creatinine, calcium, phosphate and alkaline phosphatase (ALP) concentrations were measured. Cortisol concentration was measured 1-hour after injection of ACTH (0.5 mg synacthen ,IV). Two GH provocative testing with Clonidine (0.15 mg/m2 orally) and glucagon (0.1 mg/kg IM) were performed on two occasions. After 3 days of adequate carbohydrate intake a standard oral glucose tolerance test (1.75 g/kg of glucose) was done in thalassaemic children. IGF-I generation test (293) was performed in all the patients. The test entails measurement of morning basal circulating IGF-I concentration, followed by injection of human growth hormone (0.1 mg/kg/dose, S.C.) and re-measuring IGF-I concentration next morning.

Six patients with beta thalassaemia major with growth retardation (HtSDS and GVSDS) at or below -2 for their chronological age and for their respective bone age, determined by the method of Greulich and Pyle, and defective GH release in two provocative tests were treated with human growth hormone (18 units/m2/week divided on daily S.C. doses) for one year. Their growth parameters were followed-up every three months for the whole year and IGF-I concentrations re-measured at the end of the year. Children with GHD (n=11) and those with CSS (n = 8) were treated with GH (18 units/m2/week divided on daily doses) for the same period used as controls. Oral glucose tolerance test was performed every 6 months in the GH treatment groups.

Human GH and IGF-I were measured by radioimmunometric assay.
Data are presented as mean +/- SD. Statistical analyses were performed using the ANOVA test to compare analyte concentrations among groups. The paired t test was used to compare data before versus after therapy in the same group. Wilcoxon test was

used when the data were not normally distributed. Correlations between variables of interest are examined by linear regression analysis and, when appropriate multiple regression analysis.

RESULTS: (see appendix for tables and figures)

Table 1 summarises the auxologic data of prepubertal children with thalassaemia , GHD and CSS. The HtSDS was significantly lower in children with GHD Vs children with CSS and thalassaemia. Linear GV and BMI did not differ significantly among the three studied groups. The bone age was significantly delayed in the GHD group. The upper/lower segment ratio was significantly lower in thalassaemic children than in the other two groups denoting relatively slower growth of the spine compared to the limbs in this group of patients.

The biochemical and hormonal data (table 2) showed that thalassaemic children had significantly higher concentrations of serum ferritin, ALT and bilirubin, and lower haemoglobin and haematocrit values. All the children had normal serum creatinine and albumin concentrations. Two children with thalassaemia had impaired tolerance to oral glucose (1.75 g/kg) and were excluded from the GH treatment group. Serum calcium, phosphorus and alkaline phosphatase concentrations were comparable to those for controls ruling out the diagnosis of hypoparathyroidism in any of the patients. After ACTH stimulation, circulating cortisol concentrations were significantly lower in thalassaemic children versus controls. In three thalassaemic children cortisol concentrations did not rise to 400 nmol/L. However none of them had symptoms or signs of cortisol deficiency. Thyroid function was normal in 14 out of 15 children with thalassaemia. One patient had mild hypothyroidism . His FT4= 9 pmol/ml (normal, 10-25 pmol/L) and elevated TSH of 8 uU/ml (normal 0.5- 5 uU/ml). Growth hormone testing was done after thyroxin replacement for 1 month in this boy. Fasting serum insulin concentrations did not differ between thalassaemic (12+/- 7.8 ug/L) and control group(14.5 +/- 5.8 ug/L.

Growth hormone/IGF-I/IGFBP3 data are presented in table 3. Thalassaemic children had significantly lower peak growth hormone response to provocation by clonidine and glucagon. Their circulating IGF-I and IGFBP3 concentrations were significantly lower

than those for the controls. The IGF-I and IGFBP3 responses to GH injection were significantly lower in thalassaemic patients versus controls, suggesting partial resistance to GH in these children. Their serum IGF-I concentrations after stimulation with GH were still lower than the basal circulating levels for the controls. The peak GH response to provocation and circulating IGF-I concentrations were significantly lower in thalassaemic children compared to those with CSS ($p< 0.005$). The IGF-I response to GH administration (IGF-D); (equal to the 24-h IGF-I value minus the basal value) was significantly lower in children with thalassaemia compared to the groups with CSS and GHD ($p<0.01$).

Table 4 compares growth and IGF-I data of 6 thalassaemic children with those for 8 children with CSS and 11 children with GHD. After one year of GH therapy (18 units/m2/week divided on daily S.C. doses) the GV of patients with thalassaemia (7.2 +/- 0.8 cm/yr.) was slower than that for children with CSS (9.9 +/- 1.2 cm/yr.). The increments of GV/yr. of thalassaemic patients was significantly lower compared to the other two groups. Despite slower growth in thalassaemic children, their GV was doubled (from 3.8 ٠,٦ −/+ cm/yr. to 7.2 +/- 0.8 cm/yr.). After GH therapy for one year the circulating IGF-I concentration were significantly lower in thalassaemic patients versus those for the control groups. None of the children developed impaired glucose tolerance or hypertension during treatment.

Correlation between circulating hormonal and ferritin concentrations for all the study children are presented in table 5. Serum ferritin concentration was correlated significantly (negatively) with GH peak response, IGF-I, IGFBP3 and insulin concentrations. Peak GH response was correlated significantly with IGF-I and IGFBP3 supporting the view that GH is the major regulator of both IGF-I and IGFBP3 synthesis.

DISCUSSION

The growth promoting activity of IGF-I is determined not only by the concentration of IGF-I but also by the amounts of various IGF binding proteins (IGFBPs).(105, 109) Among these IGFBP3 is the major binding protein. (314) IGFBP3 has been observed to

potentiate the effects of IGF-I in bone. (183-187,315,316) In addition, recent data clearly indicate that IGFBP3 prolongs the half life of circulating IGF-I levels and changes the clearance pattern of plasma IGF-I.(317) Current opinion favours GH as the major regulator of IGF-I and IGFBP3 in humans. In this study, the GH responses to provocation with clonidine and glucagon was impaired in short prepubertal children with beta thalassaemia major compared to those for short normal children. Ten out of the 15 children with thalassaemia did not mount an appropriate GH response (> 10 ug/L) in both provocative tests. These findings support previous reports indicating impairment of function along the hypothalamic-pituitary growth axis. (251-255) In malnourished children with decreased IGF-I synthesis the basal and stimulated GH levels are significantly high (53) indicating stimulation of their hypothalamic-pituitary axis by the low circulating concentrations of IGF-I. In thalassaemic children, the presence of normal basal GH levels despite low circulating IGF-I levels suggest defective hypothalamic-pituitary feedback mechanism. This might be secondary to defective GH secretion. Histopathological changes with significant siderosis of the pituitary gland and secondary atrophy of the somatotrophs can explain the dysfunction of this axis,(318) and the increased incidence of defective GH secretion in our children , with defective chelation therapy, compared to other studies with properly chelated patients. Impaired GH secretion can explain in part the significantly lower IGF-I and IGFBP3 synthesis with subsequent growth impairment in children with thalassaemia major. However, haemosiderosis of the liver in these patients with disturbed hepatic function might also decrease IGF-I synthesis. In our study Serum ferritin concentration was significantly correlated with IGF-I concentration ($r = -0.45$, $P < 0.05$), IGFBP3 ($r=-0.42$, $p < 0.05$) and peak GH response to provocation ($r=-0.34$, $p < 0.05$) which support the view that iron overload might affect adversely the GH/IGF-I/IGFBP3 secretion. The deficiency of IGFBP3 in thalassaemic patients might contribute to their growth impairment by decreasing the growth promoting effects of IGF-I. We (319) and others (236) reported progressive impairment of insulin secretion in children with thalassaemia due to haemosiderosis of the pancreas. Other investigators stressed as well the importance of insulin resistance in these patients. (236,237) The progressive loss of the anabolic functions of insulin might contribute to the delayed growth of these children either directly and/or through inhibition of IGF-I synthesis and function.(89,90). In our study basal (fasting) serum concentration of insulin was correlated significantly with concentrations of IGF-I ($r=0.541$, $P < 0.01$) and IGFBP3 ($r= 0.42$, $p<0.05$). Delayed or arrest of puberty is common in patients with thalassaemia (195,196) probably due to disturbed gonadotropin-releasing hormone

(GnRH) secretion (320) with consequent deficiency of sex-steroids. Sex steroids can influence growth through the modulation of IGF-I-induced cellular response. (172,176,177,179,180) and their deficiency add significantly to the growth delay and osteoporosis of thalassaemic children.(181) This might explain the relatively short upper segment, in addition to the mild vertebral changes observed in our thalassaemic group.

Malnutrition, primarily caused by inadequate nutrient intake, as indicated by the capacity to gain weight appropriately when provided with nutrition support, (215) is another correctable cause of growth delay in thalassaemic children. Malnutrition can inhibit growth through inhibition of IGF-I (53,93), IGFBP3 (321) synthesis and insulin release (322). However, our group of patients had normal BMI, mid-arm circumference and skin fold thickness, and normal serum albumin and basal GH concentrations. Analysis of their dietary intake, by using the recall method, showed normal quantitative and qualitative dietary intake. These factors collectively exclude any major role played by malnutrition in this group of children with thalassaemia.

Impaired linear growth in thalassaemic children, despite regular transfusion and desferrioxamine therapy , who have normal GH secretion suggests the possibility of GH resistance. In this study IGF-I generation test showed that these patients do not secrete adequate IGF-I after GH stimulation when compared to normal short children or those with GHD. After one year of GH therapy, despite giving the same dose/m2 to all the children, their circulating IGF-I concentrations were significantly lower than those for the short normal control group and those with GHD. These data are in concert with those of Werther GA et al (258) who reported lack of response of non-suppressible insulin-like activity to short-term administration of human growth hormone in their thalassaemic patients. In our 6 thalassaemic children treated with GH the growth velocity increased significantly from 3.8 +/- 0.6 cm/yr. to 7.2 +/- 0.8 cm/yr. (doubling). However, the increment of the rate of growth was significantly slower compared to the control groups. Collectively these findings might suggest the presence of significant GH resistance in these patients which could attenuate the growth promoting effects of GH therapy. Low LC et al (323) have shown that with higher (supraphysiological) (30units/m2/week divided on daily S.C. doses) doses of exogenous GH there has been a progressive increase of IGF-I production in their thalassaemic

patients. It is known that higher GH doses during treatment possibly elicit a higher IGF-I and GV response, however this high dose might be necessary in thalassaemic children to overcome the possible GH resistance. However, supraphysiological doses of GH might increase the risk of inducing diabetes and hypertension (324) in these high risk patients. Human IGF-I therapy , alone or in combination with GH and/ or IGFBP3 , appears to be an attractive alternative to overcome the GH resistance and avoid the high risk of developing diabetes in them. In human (325,326) and animal (316,317) experiments this combination of growth factors appear to be useful.

In summary; children with beta-thalassaemia and short stature have defective GH/IGF-I/IGFBP3 axis that might be secondary to haemosiderosis of their pituitary gland, liver and pancreas. Beside regular blood transfusion and proper chelation therapy these patients needs early management of their endocrinopathy. Treatment of their hypothyroidism, hypogonadothypogonadism , diabetes mellitus and defective GH/IGF-I/IGFBP3 axis can markedly improve their growth. In addition, these patients might have partial GH resistance that requires supraphysiological doses of growth hormone and/or human IGF-I therapy.

Study 10: SPONTANEOUS AND PROVOKED GROWTH HORMONE (GH) SECRETION AND INSULIN-LIKE GROWTH FACTOR-I (IGF-I) AND IGF-BINDING PROTEIN-3 (IGFBP3) CONCENTRATIONS IN PATIENTS WITH BETA-THALASSAEMIA AND DELAYED GROWTH.

(The Journal of Tropical Pediatrics, Accepted for publication on December 1997)

(Presented at the XXII International Congress of Paediatrics, Amsterdam, The Netherland, 9-14 August, 1998)

AIM OF THE WORK:

1. To investigate the pulsatile properties of spontaneous GH secretion;
2. to measure the circulating levels of IGF-I/IGFBP3 axis in short prepubertal children with beta thalassaemia major and;
3. to measure the pituitary size by MRI in these children.

PATIENTS AND METHODS

Seventeen children with (thalassaemia major and delayed puberty were recruited for the study from the thalassaemia clinic of the Alexandria University Children's Hospital, Alexandria Egypt for the study. All have been treated with a chronic low transfusion regimen (to keep their haemoglobin above 9g/dl) , with IM chelation treatment (3-5 times/ week) (suboptimal chelation due to economic reasons) and were taking 5 mg of folic acid per day. Fifteen age-matched children with constitutional short stature (CSS) (with height standard deviation score at or below -2 and normal GH response to provocation) and matched for the pubertal stage served as controls. Criteria for entry into the study were as follows: 1. Age > 15 and < 25 years; 2. height less than the fifth percentile; 3. Delayed puberty , defined as pubarche Tanner 2 or less, gonadarche stage in the boys of 1 and thelarche stage in the girls of 1, at the age above 14 year and 13 year respectively. None of the children had intrauterine growth retardation, severe malnutrition, diabetes, dysmorphic trait, exposure to irradiation, or any other systemic illness. Preliminary investigations included measurement of electrolytes, serum calcium, phosphate, alkaline phosphatase, ferritin, urea, and creatinine concentrations, urinalysis and complete blood cell count. Skeletal maturation was determined by the method of Greulich and Pyle (278). Informed consent was obtained from the patients and their parents before including in the study. The ethic Committee of Alexandria University has approved the protocol of the study.

Anthropometric measurements included weight (obtained to the nearest 100 g using a digital scale (Seca model 770), height (using the Harpenden scale), mid-arm circumference (using a metal tape), and triceps-skinfold thickness (using the Holtain Calliper). Growth velocity cm/yr was measured over a whole year for all the patients. Height standard deviation score (HtSDS) and body mass index (BMI) were calculated. Normal population data were according to Tanner et al (277).

After an overnight fast a venous sample was withdrawn for measurement of free thyroxin (FT4) , thyrotropin (TSH), insulin-like growth factor-I, IGFBP3 , and 7:00 - 8:00 A.M. cortisol , testosterone (T), Oestradiol (E2), LH and FSH concentrations. Two GH provocative testings with Clonidine (0.15 mg/m2 orally) and glucagon (0.1 mg/kg I.M.) were performed on two occasions after priming with ethinyl Oestradiol in females and testosterone in males. thalassaemic children with normal GH response to provocation (peak GH = or > 10 ug/L) (n = 10) underwent a 12-hour study of spontaneous GH secretion starting at 8 p.m. An indwelling catheter was inserted in a forearm vein, and a continuous blood sample was obtained in 20-min fractions through a peristaltic pump (Cormed, Medina, NY). GH peak analysis was performed visually by examining a plot of serum GH against time. For each GH profile the integrated concentration, the mean pulse amplitude, the maximum nocturnal peak, and the number of peaks above 5 ng/ml were analysed. Five randomly selected children with CSS matched for the pubertal stage and bone age, underwent similar 12-hour profiles of GH.
LH, FSH, E2 and T concentrations were measured by RIA methods, employing reagents purchased from Radium, Pomezia, Italy). The immunoradiometric assay (IRMA) on solid phase (coated tube), based on monoclonal double antibody technique, was used for LH and FSH detection. Intraassay and interassay coefficients of variations were respectively: LH 5.6 and 7.8 per cent, FSH 6.7 and 8.2 per cent, E2 5.8 and 8.7 per cent and T 6.1 and 9percent. Data are presented as mean +/- SD. Normal data for the different hormonal levels were according to published data.
Human GH and IGF-I were measured by radioimmunometric assay, employing reagents purchased from Nichols Institute (San Juan Capistrano, CA). IGFBP3 was measured by radioimmunoassay in Serono Bioscientific Laboratories and Bioscience Services employing reagents supplied by Mediagnost.

MRI study of the hypothalamic-pituitary area was performed in 10 patients with thalassaemia (five with GH deficiency, two with abnormal spontaneous nocturnal GH

release and 3 with normal GH secretion), using a gyroscan Philips 1.5.T (Tesla) machine performing the following sequences : -1. Sagittal T1,wI , 2. Coronal T1,wI for the sella tursica , 3. Axial T1 wI for the whole brain, 4. Axial T2 wI over the pituitary and hypothalamic regions 5. Sagittal T2 wI for the brain. The field of view ranged between 180 mm for the pituitary region to 220 mm for the whole brain. Slice thickness was 3 and 6 mm and interslice gap = 0.3 and 0.6 mm for the pituitary and the whole brain respectively. Data are presented as mean +/- SD. A control group consisted of 10 aged between 14 and 23 years with HtSDS = 0.5+/- 0.3 were used as controls for the evaluation of pituitary size (5 males and 5 females). None of them had received cranial radiation therapy and they had all been referred for MRI for various neurological reasons.

Statistical analyses were performed using the unpaired t test to compare analyte concentrations among groups. Wilcoxon test was used when the data were not normally distributed. Correlation between variables of interest are examined by linear regression analysis.

RESULTS: (see appendix for tables and figures)

Table (1) summarises the auxologic data of thalassaemic patients and controls. The HtSDS, BMI, and GV did not differ significantly among the two groups.

The biochemical and hormonal data (Tables 2,3) showed that thalassaemic children had significantly higher concentrations of serum ferritin, ALT and bilirubin and lower haemoglobin values versus the control group. Children of both groups had normal serum T4, TSH , 8:00 A.M. cortisol, creatinine, albumin, calcium, phosphate and ALP concentrations. The circulating concentrations of IGF-I and IGFBP3 were significantly decreased in thalassaemic patients versus controls (table 3). The peak GH response to provocation by clonidine and glucagon was significantly low in thalassaemic patients. Seven , out of the 17, patients with thalassaemia had classic GH deficiency (peak GH < 7 ug/L after provocation by clonidine and glucagon, with low IGF-I , and IGFBP3 concentrations).

These 10 patients underwent 12-h study of nocturnal GH release (figure 1). Analysis of the pulsatile properties revealed that the integrated and mean GH concentrations over

12h were markedly lower in thalassaemic patients versus controls . Five out of the ten studied patients had maximum nocturnal GH peak below 10 ug/L and four of them had mean nocturnal GH concentration below 2 ug/L. Two had severe neurosecretory dysfunction of GH secretion (patient 6, 10). Their circulating IGF-I and IGFBP3 concentrations were markedly reduced compared to those for controls.

Correlation between circulating hormonal and ferritin concentrations for all the study children are presented in table 5. Serum ferritin concentration was correlated significantly (negatively) with mean nocturnal GH , IGF-I, IGFBP3 and insulin concentrations. Mean nocturnal GH concentration and the peak nocturnal GH level were correlated significantly with IGF-I and IGFBP3 supporting the view that GH is the major regulator of both IGF-I and IGFBP3 synthesis.

MRI studies revealed marked reduction of the pituitary volume in thalassaemic children (305 +/- 125 mm3) versus age-matched normal controls (618 +/- 87 mm3) (p<001). Those patients with GH deficiency had variable abnormalities including: complete empty sella (n= 2) , marked diminution of the pituitary size (n=4) , abnormal signal pattern of the gland (n=4) and thinning of the pituitary stalk (n=3) with its posterior displacement (n=2) (figure 2). None of the control children had abnormalities of the pituitary gland or its stalk.

DISCUSSION

The growth promoting activity of IGF-I is determined not only by the concentration of IGF-I but also by the amounts of various IGF binding proteins (IGFBPs).(327,328) Among these IGFBP3 is the major binding protein. (329) IGFBP3 has been observed to potentate the effects of IGF-I in bone. (315,316,330) In addition, recent data clearly indicate that IGFBP3 prolongs the half life of circulating IGF-I levels and changes the clearance pattern of plasma IGF-I.(317) Current opinion favours GH as the major regulator of IGF-I and IGFBP3 in humans. In this study, the GH responses to provocation with clonidine and glucagon was impaired in short prepubertal children with beta thalassaemia major compared to those for short normal children. Seven out of the 17 children with thalassaemia did not secrete GH after provocation (peak GH <7 ug/L) in both provocative tests. Five of the children had normal GH response to provocation , but abnormal nocturnal spontaneous GH secretion. MRI studies of the

hypothalamic-pituitary area of 12 patients with thalassaemia (7 with GH deficiency, 2 with abnormal spontaneous nocturnal GH release and 3 with normal GH secretion) revealed complete empty sella (n= 2) , marked diminution of the pituitary size (n=4) , abnormal signal pattern of the gland (n=4) and thinning of the pituitary stalk (n=3) with its posterior displacement (n=2) in those patients with defective GH secretion (Fig 2) with relatively normal hypothalamic area, proving a good correlation between the structural alterations of the pituitary gland and dysfunction/deficiency of GH secretion in these patients. In support to our data previously published autopsy studies showed marked histopathological changes with significant siderosis of the pituitary gland and secondary atrophy of the somatotrophs (318). The increased incidence of defective GH secretion in our thalassaemic children, compared to other studies (252-255), could be explained on the basis of incomplete iron chelation in our patients with more siderosis of the pituitary gland.

All our patients with thalassaemia had significantly lower concentrations of circulating IGF-I and IGFBP3. Impaired GH secretion can explain in part the significantly lower IGF-I and IGFBP3 synthesis with subsequent growth impairment in children with thalassaemia major. However, haemosiderosis of the liver in these patients with disturbed hepatic function might also decrease synthesis of IGF-I and IGFBP3. In our study serum ferritin concentration was significantly correlated with IGF-I concentration ($r = - 0.47$, $P< 0.01$), and IGFBP3 ($r=-0.43$, $p< 0.01$). This supports the view that siderosis of the liver can adversely affect the IGF-I/IGFBP3 synthesis. Deficiency of IGFBP3 in thalassaemic patients might contribute to their growth impairment by decreasing the growth promoting effects of IGF-I.

We (319) and others (236) reported progressive impairment of insulin secretion in children with thalassaemia due to haemosiderosis of the pancreas. Other investigators stressed as well the importance of insulin resistance in these patients. (326,237). Insulin deficiency and/or resistance might be a contributing factor compromising the growth potential of these children through inhibition of IGF-I synthesis and/or secondary to increased production of inhibitory IGF-binding proteins. In this study, although the fasting serum insulin concentrations were within the normal range, they were correlated significantly with circulating IGF-I and IGFBP3 levels.

Delayed or arrest of puberty is common in patients with thalassaemia (195,196) due to disturbed gonadotrophins secretion (320) with consequent deficiency of sex-steroids.

Sex steroids levels were low in all our patients compared to normal data at the same pubertal stage. This can influence growth through the modulation of GH secretion and IGF-I-induced cellular response (172,176,177,179,180) and consequently impairs linear growth and attenuates the pubertal growth spurt in thalassaemic children (181).

The finding of impaired linear growth in thalassaemic children, despite regular transfusion and desferrioxamine therapy, who have normal GH secretion suggests the possibility of GH resistance. In a previous report our group studied IGF-I generation in a group of thalassaemic patients (n=15) and found that they do not secrete adequate IGF-I after GH stimulation when compared to normal short children or those with growth hormone deficiency (GHD). After one year of GH therapy, despite giving the same dose/m2 to all the children, thalassaemic children had significantly lower IGF-I concentrations versus those for the short normal control group and those with GHD.(331) These data are in concert with those of Werther GA et al (258) who reported lack of response of non-suppressible insulin-like activity to short-term administration of human growth hormone in their thalassaemic patients. We treated 6 thalassaemic children with GH for a year. Their growth velocity increased significantly from 3.8 +/- 0.6 cm/yr. to 7.2 +/- 0.8 cm/yr. (doubling). However, the increment of the rate of growth was significantly slower compared to a control group with CSS. Collectively these findings suggest the presence of significant GH resistance in these patients, which could attenuate the growth promoting effects of GH therapy. In concert with our view, Low LC et al (323) have shown that with higher (supraphysiological) (30units/m2/week divided on daily S.C. doses) doses of exogenous GH there has been a progressive increase of IGF-I production in their thalassaemic patients. Although it is known that higher GH doses during treatment possibly elicit a higher IGF-I and GV response, however this high dose might be necessary in thalassaemic children to overcome the possible GH resistance. However, supraphysiological doses of GH might increase the risk of inducing diabetes and hypertension (324) in these high-risk patients. Human IGF-I therapy, alone or in combination with GH and/ or IGFBP3 , appears to be an attractive alternative to overcome the GH resistance and avoid the high risk of developing diabetes in them. In human (325,326) and animal (316,317) experiments this combination of growth factors appear to be useful.

In summary; children with beta-thalassaemia and short stature have high incidence of abnormalities involving the GH/IGF-I/IGFBP3 axis with low circulating IGF-I and IGFBP3 concentrations. Decreased GH response to provocation (7/15) and abnormal

properties of spontaneous nocturnal GH secretion (5/15) have been detected in this study and appear to be important etiologic factors. In these patients defective GH secretion is associated with abnormal structure of the pituitary gland and its stalk. These changes might be secondary to haemosiderosis of the pituitary gland. However, children with thalassaemia who have normal GH response to provocation and normal spontaneous (mean and integrated) GH secretion still have low circulating IGF-I concentrations suggesting partial resistance to GH. Early treatment of defective GH/IGF-I/IGFBP3 axis in these children might markedly improve their linear growth, however, these patients usually require supraphysiological doses of growth hormone.

Study-11: BONE MINERAL DENSITY IN PREPUBERTAL CHILDREN WITH BETA-THALASSEMIA: CORRELATION WITH GROWTH AND HORMONAL DATA.

Metabolism 1998; 47 (5): 541-548.

AIM OF THE WORK:

1. To measure the bone mineral density and;
2. to investigate some factors affecting bone mineral metabolism in 30 children with beta thalassaemia major and attempt to find a relationship, if any, between the degree of siderosis, calcium-phosphate balance, GH/IGF-I/IGFBP3 axis, parathormone(PTH) secretion and auxologic data on the one hand and BMD on the other hand.

PATIENTS AND METHODS

Thirty-three prepubertal patients with b thalassaemia major randomly selected from those attending the outpatient Paediatric Haematology Clinic of Alexandria University Children's Hospital, Alexandria, Egypt, were the subjects of this study. All children were on regular blood transfusion to keep their haemoglobin (Hb) concentration above

10g/dl. All were on folic acid supplements and iron chelation with daily IM desferioxamine. Fifteen age-matched normal short children (CSS) (height SDS at or below -2, annual growth velocity at or below 5cm/yr., with normal GH response to provocation and delayed bone age) served as controls. None of the children had history of intrauterine growth retardation, any other systemic or endocrine disease, dysmorphic trait or CNS irradiation. All had normal tolerance to oral glucose load (1.75 g/kg of dextrose). Three patients who had abnormal glucose tolerance were excluded from the study. Informed consent for the testing procedures was obtained from the parents and when appropriate from the children before entering into the study. The protocol of the study was approved by the ethics committee of Alexandria University. All children were examined with special emphasis on nutritional data. The auxologic data included weight, height and mid-arm circumference. Harpenden's callipers and anthropometric measurements were used. The data recorded were the average of 3 sequential measurements determined by the same observer (ATS). The height standard deviation score (HtSDS) and body mass index (MBI) were calculated and recorded. The linear growth velocity (GV) cm/yr. was calculated for the last year. Normal population data were according to Tanner et al. (277) The bone age was determined according to Greulich and Pyle atlas.(278) On the day of admission, venous blood samples were obtained for determination of complete blood count (CBC), and serum concentrations of albumin, bilirubin, and alanine transferase (ALT) concentrations. Following an overnight fast (8-h) venous blood samples were withdrawn through a polyethylene catheter inserted in a forearm vein between 8 and 9 AM. The serum was separated from the formed elements by centrifugation and kept frozen at -20 c until analysed for GH, IGF-I, IGFBP3, free thyroxin (FT4), thyrotropin (TSH), cortisol, PTH (intact molecule), calcium (Ca), phosphorus (PO4), alkaline phosphatase (ALP), ferritin, interleukin-1-B (IL-1-B) and Tumour-necrosis factor-alpha (TNT-A) concentrations. After obtaining the basal samples, an oral dose of clonidine (0.15 mg/m2) and iv. dose of ACTH (SYNACTHEN) 1ug/m2 were given and blood samples collected every 30 min for 2 hours for measurement of GH and after 60 min for cortisol. On the next morning a standard glucagon test for GH release was performed.

Human GH and IGF-I concentrations were measured by radioimmunometric assay. IGFBP3 was measured by radioimmunoassay in Serono Biochemical laboratories (SCL) & Bioscience Services employing reagents supplied by Mediagnost. Intact PTH was measured in the serum using an immunochemiluminometric method. IL-1-B and

TNF-A were measured using ELISA technique (BIOKINE, T-Cell Diagnostics, Cambridge, MA.

The bone mineral density (BMD) of the lumbar spine (2nd,3rd and 4th lumbar vertebrae) were measured by dual photon absorptiometry (DPA) using a Norland 2600 bone densitometer. All children were scanned in the supine position. BMD data were expressed in g/cm2 and were compared with BMD of normal children of the same age. (327)

Statistical analyses were done using the unpaired t-test to compare mean analyte concentrations among the two study groups when the data were normally distributed and Wilcoxon test when they were not. Statistical significance was accepted at $p < 0.05$. Multiple regression analysis was performed using BMD as the dependent variable and all the other auxologic and biochemical data as the independent variables. Data are presented as mean +/- SEM.

RESULTS: (see appendix for tables and figures)

The anthropometric and bone age data are presented in "table 1". The chronological age, HtSDS, GV, BMI and bone age did not differ significantly between the two study groups. The biochemical, hormonal and BMD data are shown in "tables 2,3". The circulating concentrations of albumin, creatinine, ALP, and PO4 did not differ significantly among the 2 groups. Hypocalcaemia {Ca = or < 1.4 nmol/L (5.7 mg/dl)} was detected in 5 patients. Three of them (aged 11, 12.5 and 14 years) had other biochemical evidence of hypoparathyroidism (high PO4, normal ALP, and low PTH concentrations). The other two patients (aged 9 and 11 years) had biochemical evidence of rickets (low PO4, high ALP and PTH and low 25-hydroxy vitamin D3 concentrations). The circulating concentrations of IGF-I and IGFBP3 were significantly lower in thalassaemic children compared with controls. Their peak GH responses to provocation with clonidine and glucagon were significantly below those for controls. Twelve out of the 30 children with b thalassaemia did not mount GH response to provocation above 7 ug/L. Although basal levels of cortisol did not differ between the two groups, cortisol response to low dose ACTH test was significantly lower in thalassaemic children versus controls. Two children had mild chemical hypothyroidism

(FT4 = 9.5 and 8.7 pmol/L and TSH = 8.6 and 10.3 uIU/ml). Both were treated with L-thyroxin for a month before testing their GH response to provocation.

Dual photon absorptiometry revealed that children with b thalassaemia had significant reduction of their BMD (30% less) compared to average BMD for age and sex matched normal children, corresponding to BMD = -1.5 to -2 SD. Thalassaemic children had significantly lower BMD versus age-matched children with CSS. Correlations between BMD and different parameters are shown in "Table 4" and "Figure1". BMD was correlated significantly ($p<0.01$) with age, height, weight and BMI as well as with the circulating concentrations of IGF-I and IGFBP3. No significant correlations was found between BMD on the one hand and PTH, PO4, Ca, or ALP concentrations on the other hand. IL-1-B and TNF-A concentrations did not differ significantly between the thalassaemic group (25.9 +/- 11.4 pg/ml and 399 +/- 113 pg/ml respectively) and controls (21.1 +/- 6.4 pg/ml and 383 +/- 122 pg/ml).

DISCUSSION

From infancy through late adolescence the activity of bone forming exceeds bone resorption, resulting in a steady accumulation of bone mass. On average, most of the skeletal mass is accumulated by the age of 18 years.(158,161) Since the bone mass is one of the main determinants of fractures, high bone mass at skeletal maturity (peak bone mass) is considered the best protection against age-related bone loss.(163) Small differences in bone mass at skeletal maturity of 5-10% could contribute to substantial differences in the incidence of osteoporotic fractures.(164)

Bone modelling and skeletal consolidation result from a complex sequence of hormonal changes in interaction with nutritional factors, where the concerted actions of GH, IGF-I and sex hormones and their receptors, besides other factors, are responsible for timing and attainment of skeletal consolidation. At puberty, circulating IGF-I concentrations correlate with sexual development. Specifically, the surge in sex steroids in turn increases the secretion of growth hormone, which stimulates the production of IGF-I (166,328) and increases bone mass (172). In addition, a large number of other factors interact at the level of osteoblast, osteoclast and other cells to regulate the balance between net resorption and formation. These include parathormone (PTH), vitamin-D, and cytokines.(172)

The GH/IGF-I/IGFBP3 axis and factors affecting it:

In this study the hormonal profile of children with b thalassaemia showed significant deficiency of their circulating IGF-I and IGFBP3 (both are GH dependent peptides). The significant correlation between IGF-I levels and HtSDS and BMD supports a major role played by IGF-I in stimulating linear growth and bone mineralisation.

Forty percent of our prepubertal thalassaemic children had defective GH secretion after provocation by clonidine and glucagon. In concert with our findings Danesi et al (329), Saglamer et al (330) and Pintor et al (253) reported low GH response to provocation by insulin hypoglycaemia, arginine, L-dopa, and GH-releasing hormone in many of their patients denoting impairment of somatotroph function. This can explain low IGF-I synthesis in some patients. However, our thalassaemic children with normal GH secretion had low circulating IGF-I concentrations (50 +/- 19 ng/ml) comparable to those with defective GH release (46 +/- 24 ng/ml) suggesting that other factors contribute to low IGF-I synthesis in these children. Leger et al (331) found that decreased IGF-I secretion occurs before alteration in GH secretion in response to GH-releasing hormone, arginine or insulin. Other investigators reported neurosecretory dysfunction of GH secretion to be responsible for decreased IGF-I synthesis in some of those patients with normal GH response to provocation (255,256). Some investigators indicated that decreased GH secretion may be due to an age-related deterioration of the hypothalamic-pituitary function secondary to progressive siderosis. (331,252) In support, Perignon et al (332) reported that their patients with b thalassaemia had low IGF-I concentration which did not increase at puberty. Although the idea of a defect at the hepatic GH receptor or postreceptor level was suggested to explain low IGF-I production (26), Postel-Vinay et al (307) found no evidence for a defect in GH binding to liver membranes in thalassaemic patients.

It is well recognised that nutritional status has an important influence on the GH/IGF-I/IGFBP3 axis. (333) Fasting results in increased GH secretion and decreased IGF-I levels (334) and proper nutrition increases IGF-I levels in malnourished children.(53). Our children with thalassaemia had BMI and MAC at or below 10th centile for age and sex suggesting a mild degree of undernutrition. Decreased food intake(215), pancreatic exocrine dysfunction, hepatic cirrhosis (14,217) and /or hypermetabolism secondary to bone marrow hyperactivity and increased cardiac work might compromise nutrition and growth in these children. In this study circulating IGF-I concentrations were

correlated significantly with alanine transferase levels (r= -0.465, p <0.01), and 50% of our children were hepatitis-B surface antigen carriers and had significantly elevated ALT concentrations. Clinically, 25 out of the 30 patients had cirrhotic livers. In one study (215) nutrition intervention resulted in improvement of weight for height and increased IGF-I concentration. In malnutrition (53) and hypercatabolic states (280) the low IGF-I production is associated with high basal and stimulated GH levels denoting normal sensitivity of the hypothalamic pituitary axis to the low IGF-I level (normal feed-back). In thalassaemia the low-normal GH levels despite the low circulating levels of IGF-I proves defective feedback effect of decreased IGF-I on the pituitary (either due to lack of sensitivity or defective somatotroph function). We and others reported partial resistance to GH in thalassaemic children evidenced by low IGF-I generation in response to exogenous administration of GH and slow linear growth on GH therapy (323,335-336)

During normal pubertal growth spurt sex steroids increase GH secretion with subsequent increase of IGF-I levels. Sex steroids and GH each contribute approximately 50 per cent of the height gain. Children with GH insufficiency not treated with exogenous GH attain only 50 to 66 per cent of the expected growth spurt (43,124,125,136). Reduction of sex-steroid concentration during gonadotrophic-releasing hormone therapy decreases GH secretion and serum IGF-I concentration (48,137,337). Patients with b thalassaemia have a high incidence of failure of puberty (51 per cent of boys and 47 per cent of girls) and secondary amenorrhoea (23 per cent).(195) Defective gonadotropin secretion with subsequent sex-steroid deficiency have been detected in these patients with low IGF-I levels.(193,197,338) Even those who enter puberty do not have the enhanced GH secretion and increased IGF-I synthesis pattern accompanying normal puberty (252,332) denoting a major effect of sex-steroid deficiency on the GH/IGF-I axis in peripubertal and pubertal children with b thalassaemia. Unlike children with constitutional delay of puberty who secrete normal GH in response to provocation after priming with sex steroid (43), in our prepubertal thalassaemic patients above the age of 12 years (n=8) the peak GH response to provocation did not improve after (7.5 +/- 2.1 ug/L) versus before priming with oestrogen (6.5 +/- 1.5 ug/L).

The reported prevalence of diabetes mellitus in treated b thalassaemia is about 16 per cent, while the incidence of impaired glucose tolerance approximates 60 per cent. Islet cell destruction secondary to iron overload and/or exhaustion of B-cells due to long-

term insulin resistance and liver derangement are possible pathogenic factors. (224,235,232,307) Defective insulin secretion and insulin-resistance state can impair hepatic IGF-I production (339,93). Moreover, insulin plays an important part in determining the bioavailability of IGF-I through its action on IGFBP1. Therefore, defective insulin secretion or insulin resistance in thalassaemic children can increase hepatic production of IGFBP1 leading to decreased bioavailability of IGF-I (90,340). However, this factor can be excluded in our patients with normal glucose tolerance.

In summary, the markedly decreased hepatic production of IGF-I in our thalassaemic prepubertal patients with normal glucose homeostasis can be attributed to defective GH secretion , hepatic cirrhosis (secondary to siderosis and/or chronic viral hepatitis), and/or GH resistance. The delayed or lack of pubertal development and the occurrence of IDDM with advancing age, might further impair the secretion and/or the bioavailability of IGF-I.

IGF-I is a potent stimulator of linear growth and major determinant of bone mineralisation. Exogenous administration of IGF-I has been shown to increase growth and bone formation in human and animals This effect is potentiated when IGF-I is combined with IGFBP3 (316,317,325,326). In our children with beta thalassaemia the decreased production of IGF-I and IGFBP3 and the significant correlation between these growth factors and BMD and linear growth (weight, height and HtSDS) parameters suggested a major role played by them in the pathogenesis of osteoporosis. GH or IGF-I therapy may improve linear growth and bone mineralisation in these children as seen in patients with GH deficiency/resistance and other diseases with osteopenia.(341-343) This hypothesis needs to be tested by double-blind therapeutic trial of GH or IGF-I in these patients.

Calcium-phosphate balance and PTH:

In this study hypocalcaemia occurred in 5 out of 30 children with b thalassaemia. All the 5 children had markedly decreased BMD. Analyses of the other biochemical parameters differentiated 2 possible disease entities. Two out of the 5 patients had evidence of hypoparathyroidism (low Ca, high PO4, normal ALP, and low PTH). This can be explained by siderosis of the parathyroid gland (263-344-346) In support to this view, Gertner et al (347) found low PTH reserve to induced hypocalcaemia in their thalassaemic patients. The other 3 patients had biochemical evidence of rickets (low PO4, low Ca, high ALP, high PTH, and low 25-hydroxy vitamin D3). In concert with

this finding, De Vernejoul et al (348) reported osteomalacia in their thalassaemic patients. The cause of rickets/osteomalacia in these patient is defective 25-hydroxylation due to hepatic impairment and/or decreased vitamin-D absorption in these children. Impaired osteoblast function with diminished bone formation and low serum concentration of 25-hydroxy vitamin D3 with high PTH levels have been reported in patients with hemochromatosis and liver cirrhosis and in pigs overloaded with parenteral iron. (348-352)

The normocalcaemic (25/30) patients with b thalassaemia had slightly higher PTH concentration and significantly lower BMD versus normal children. In agreement with this finding Pawlotsky Y et al (353) reported elevated PTH concentration, normal serum calcium level and increased bone resorption in their haemochromatic patients. It appears that both vitamin-D deficiency and hypoparathyroidism might affect bone mineralisation in thalassaemic children.

Cytokines:

Cytokines represent a group of factors influencing the balance between bone formation and resorption. Increased bone resorption induced by an overproduction of critical cytokines, such as IL-1, TNF and GM-CSF by the hyperactive marrow cells and monocyte/macrophage lineage, is an attractive theory to explain the pathogenesis of osteoporosis seen in patients with b thalassaemia, as with other diseases. IL-1 and TNF are among the most powerful stimulators of bone resorption known and are well recognised inhibitors of bone formation.(353-355) However, we found normal serum levels of IL-1 and TNF in our thalassaemic patients comparable to those for control children which might rule out a significant role played by these cytokines in the production of osteoporosis in these children.

Cortisol secretion:

In this study children with beta thalassaemia had significantly lower cortisol response to provocation with low-dose ACTH. Other studies reported both low (225,356) and normal cortisol concentrations and response to high-dose ACTH. (238, 269, 270, 357, 358) Slate grey pigmentation which becomes progressively intense with time, poor weight gain, weakness and absent adrenarche were significant signs in our thalassaemic patients. However, the contribution of different factors including adrenal insufficiency , siderosis and anaemia in the production of these manifestation is difficult to assess. Macintosh et al (225) reported high circulating ACTH in beta thalassaemia and

suggested that it is the cause of the pigmentation. In concert with these findings, the graded dose adrenal cortical stimulation showed significant suppression of cortisol secretion. Although iron deposition in the adrenals might be the cause of adrenal insufficiency, recently it has been shown that IGF-I enhances the steroidogenesis and ACTH responsiveness of human adrenocortical cells in culture.(359-361) Deficiency of IGF-I synthesis in beta thalassaemia might contribute to the defective cortisol production and possibly other adrenal androgens which might explain the lack or delayed adrenarche in thalassaemic patients. These data suggest that replacement with physiological doses of hydrocortisone might improve some of the manifestations of the disease. In addition, increasing IGF-I level might also improve the secretion of adrenal androgens necessary for adrenarche.

The question is what possible therapeutic or preventive options are available that might influence bone mineralisation and growth in thalassaemic children? The data from this study supports the development of a controlled clinical trial to evaluate several possible therapeutic interventions. In addition to proper and aggressive nutritional intervention , which should be an integral part of any treatment strategy, possible new therapeutic interventions would include: 1. GH and/or IGF-I replacement therapy, especially for those with GH and/or IGF-I insufficiency. These measures might increase the circulating IGF-I level and consequently increase bone formation and prevent osteopenia. Adding IGFBP3 to IGF-I and/or GH therapy might potentiate their effect on bone growth and mineralisation.(341 342,343) 2. Treatment with vitamin D or vitamin D analogues in the modest doses (800-1500 IU/day of vitamin-D3) may offer a safe and substantial contribution to the prevention of osteoporosis in these children. Positive correlation of vitamin-D levels with BMD of the vertebrae and proximal femur have been found in young and old women with poor vitamin-D status.(361-363) Those patients with osteoporosis and biochemical evidence of rickets need higher doses of vitamin-D3 or its analogues for treatment. 3. Calcium supplementation which has been shown to increase bone density in the normal prepubertal children is another good potential option.(364) 4. The initiation of puberty at an appropriate age through the use of progressively increased doses of androgens or oestrogen . These agents would prevent osteoporosis and increase their BMD,(172,365-367) putting in consideration the risk of advancing the bone age faster than the height age.

In summary, prepubertal children with beta thalassaemia and normal glucose tolerance have decreased BMD, delayed growth and a defective GH/IGF-I axis. Biochemical

evidence of hypoparathyroidism or rickets may be detected in thalassaemic patients with hypocalcaemia. It is logical to propose that treatment of these patients with GH and/or IGF-I with aggressive nutritional support and supplementation with vitamin-D and/or calcium might improve their bone density and prevent the development of osteoporosis and subsequently fractures.

Study 12: DECREASED BONE MINERAL DENSITY IN PREPUBERTAL CHILDREN WITH SICKLE CELL DISEASE: CORRELATION WITH GROWTH PARAMETERS, DEGREE OF SIDEROSIS AND SECRETION OF GROWTH FACTORS.

(Accepted for publication in the Journal of Tropical Pediatrics, in press)

AIM OF THE WORK:

Patients with sickle cell disease appear to be at higher risk for developing skeletal abnormalities with significant radiological bone changes (273).
We measured bone mineral density in relation to linear growth and secretion of growth factors (growth hormone (GH), insulin-like growth factor-I,(IGF-I), and IGF-binding protein-3 (IGFBP3)) in 28 prepupertal children with SCD.

PATIENTS AND METHODS

Twenty eight prepupertal children with SCD were the subject of this study. They were randomly selected from the 162 children with SCD attending the Haematology out-patient clinic of the Royal Hospital ,Muscat, Oman. All have been on folic acid supplement and vaccinated against pneumococci. None of them had history of intrauterine growth retardation, any other systemic or endocrine disease, dysmorphic trait or central nervous system irradiation. Informed consents were obtained from all the parents and when appropriate from the patients before including in the study. Twenty five age matched children with ISS served as controls. The protocol of the study was approved by the ethical committee of SQU. All the children were examined with special emphasis on the nutritional data. The auxological measurements included weight, mid-arm circumference and scapular, triceps and abdominal skin fold thickness. Harpenden's callipers were used. Height standard deviation scores were calculated according to the formula HtSDS =(X1-X2)/ SD where X2 and SD are matched population mean height and ýSD respectively and X1 is the subject height. The body mass indices (BMI) were calculated according to the formula BMI= weight(kg)/Ht (m)2. The bone age was determined according to the Greulich and Pyle atlas (278).

After an overnight fast (8 h) venous blood samples were obtained between 8 and 9 a.m. for determination of complete blood picture , serum albumin, calcium, phophates and alkaline phosphate concentrations. The serum was separated from the formed elements by centrifugation and kept frozen at -20 c until analysed for GH, IGF-I and IGFBP3

radioimmunoassay. GH provocation test was performed using oral clonidine (0.15 mg/m2), and for those who did not respond (peak GH below 10ug/L) GH stimulation with glucagon 0. \mg/kg IM was performed . Human GH and IGF-I were measured by radioimmunometric assay, employing reagents purchased from Nichols Institute (San Juan Capistrano, CA).

Lumber spine (L-2,L-3,L-4) bone mineral density was measured in all patients by dual photon absorptiometry (DPA) using a Norlad 2600 bone densitometer. All patients were scanned in the supine position . BMD data were expressed in g/cm2 and were compared with the BMD of controls and normal children of the same age(20).The densitometer was calibrated daily using a phantom standard. the examination was performed with the child supine, knee flexed and calves resting on a cushion. These manoeuvres separated he spinous processes and prevented overlap of bony structures. All clothing that could produce artefacts was removed. No sedation or restraints were used. The scan was approximately 15 minutes. All scans were analysed with the assistance of an edge detection program in the scanner software. To minimise variability, only one experienced observer (H.B) performed all scan analyses . The coefficient of variation on repeated scan analyses was 0.7%. The L-2:L-4 spinal BMD was expressed in grams per square centimetre.

Data are presented as mean +SD. Correlation coefficients were calculated for each variable against BMD using single and multiple regression analyses were performed using BMD as the dependent variable.

RESULTS

The table presents the data of the patients and controls. According to the peak GH response to provocation , the SCD of the patients were divided into (group I) with defective GH release (GHD) and (group II) with normal GH release after provocation. The chronological and bone age did not differ significantly among the 3 study groups. SCD children had significantly lower IGF-I and IGFBP3 concentration versus controls. Those with defective GH secretion had lower IGF-I &IGFBP3 concentration compared to those with normal GH peak after provocation. The per cent BMD (measured as % of the normal values for age and sex matched children)(20) was significantly lower in children with SCD versus controls. The loss of more than 20% of

the BMD corresponds to loss of more than 1 SD of bone density. The BMD of children with SCD+ GHD was significantly lower than those with normal GH secretion. BMD was correlated significantly with the weight (r=0.547),height (r=0.52) HtSDS (r=0.369),BMI (r=0.409) and circulating IGF-I (r=0.469) and IGFBP3 (r=0.36). No significant correlation was found between BMD and serum concentration of Ca (r=0.077), PO4 (r=0.027) ALP (r=0.023), or ferritin (r=0.25,p=0.08).

DISCUSSION

This study demonstrates that children with SCD have significant reduction of BMD. The reduction of BMD of this extent (1 SD below the mean) probably represents a 50 to 100 per cent increase in the incidence of fractures (368). We attempted to study some factors that might affect BMD in those patients with SCD including, auxologic data, the degree of siderosis, calcium-phosphate balance and GH/IGF-I/ IGFBP3 axis.

In this study children with SCD had low BMI (20 out of 28 had BMI below 10^{th} centile for age and sex) and HtSDS (18 out of the 28 had HtSDS below -2). The significant correlation between BMD on the one hand and weight, height and BMI on the other hand suggests that slow growth is an important factor in the production of low BMD in these children. Delayed sexual maturation, a known feature of SCD, can significantly add to the problem by decreasing the peak bone mass(369,370)
Twelve out of the 28 children with SCD had defective GH secretion after provocation, and as a group (n=28), sicklers had significantly lower circulating concentration of IGF-I and IGFBP3 versus control group. Children and adults with GHD have reduced BMD and bone formation (371,372).In those patients, GH replacement increases bone turnover and increases BMD within 6-12 months (373).Children with SCD and GHD had significantly lower IGF-I and IGFBP3 concentrations and BMD compared with those with normal GH secretion .IGF-I, is a GH-dependent polypeptide, that circulates in the plasma preferentially bound to the high molecular weight IGFBP3 complex which acts as a reservoir prolonging the 1/2 life of IGF-I and targeting the IGFs to the relevant target organ. The bioactivity of the circulating IGF-I is therefor very much dependent on the presence of IGFBP3 in the circulation (314,374,375). IGF-I is a potent stimulator of bone formation and exogenous administration of IGF-I has been shown to increase bone formation in human and animals (376,377). This effect is potentiated when IGF-I is combined with IGFBP3 (317,378). Current opinion favours GH as the major regulator of IGF-I and IGFBP3 levels in humans (295,341). In addition , serum

levels of IGF-I and IGFBP3 are positively related to nutritional status (326,379). GH therapy increases both IGF-I and IGFBP3 (341). Therefore GH replacement and/or IGF-I therapy might improve growth and BMD in children with SCD (379).

In this study, the normal Ca , PO4 and ALP concentrations excludes an important role, if any, of disturbed Ca/PO4 balance in the production of low BMD. The degree of siderosis, evidenced by circulating ferritin concentration., was not correlated significantly with BMD. However the excess iron might inhibit Ca deposition in the bone either directly and/or through decreased hepatic synthesis of IGF-I.

The question is what possible therapeutic or preventive options are available that may influence bone mineralisation in SCD. Obviously proper and aggressive nutritional intervention is necessary to improve growth. Calcium supplements that have been shown to increase bone density in the normal prepupertal children is a potential option (379). Growth hormone and/or IGF-I therapy, especially for those with GHD and/or low IGF-I concentration , is a reasonable replacement (341,380).The initiation of puberty at an appropriate age, using oestrogen or androgens, is another option, putting in consideration the risk of advancing the bone age faster than the height age.

In summary, prepupertal children with SCD have decreased BMD, delayed growth and defective GH/IGF-I/IGFBP3 axis. It is logical to propose that treatment of these patients with GH and/or IGF-I will improve their linear growth and bone formation.

SUMMARY

This study evaluated growth and pubertal development in a large cohort of patients with β thalassaemia (n =72) and SCD (n=110), and investigated the different factors affecting them in these patients. Linear growth was significantly impaired in children with thalassaemia and SCD. Forty nine percent of thalassaemic children and twenty seven percent of the sicklers had HtSDS below -2. Eighty three per cent of the thalassaemic children and sixty seven per cent of children with SCD had GVSDS below -1. Pubertal development showed marked retardation in both group of patients. Only 27% of thalassaemic boys above the age of 14 years had testicular volume of 3 ml or more. Spontaneous menarche and breast development (B2 or more) occurred only in 26 and 42per cent of thalassaemic girls above the age of 13 years respectively. Secondary amenorrhoea occurred in two thirds of those who had spontaneous menarche. Only 50 per cent of girls with SCD above the age of 13 years had spontaneous menarche. Boys with SCD, aged 14 years or more, had significantly small testicular volume and 22.3 per cent did not show testicular enlargement (volume > 3 ml).
Retardation of growth and puberty was more marked in thalassaemic children compared with those with SCD. Thalassaemic children had significantly higher circulating ferritin concentrations and lower IGF-I levels compared to children with SCD. Linear growth velocity (cm/yr.) was correlated negatively with serum ferritin concentration in all the study children. These data denoted that excessive iron overload has a major role in the aetiology of delayed growth and impaired pubertal development in these patients. Moreover, the delayed and/or failure of puberty in thalassaemic and sickler children increases growth impairment through the lack of the synergistic effect of sex steroids on pubertal growth spurt. The negative correlation between HtSDS and age of these patients as well as the disturbed upper/lower segment ratios supported this view.

The hepatic functions and prevalence of HBS antigenaemia and HCV antibody in children with β thalassaemia and SCD were studied. Children with thalassaemia had significantly high prevalence of HBS antigenaemia and HCV antibody positivity (44.4 and 23.5 per cent) compared to normal age matched children. They had markedly elevated serum ALT concentrations and increased liver span. ALT concentrations were correlated significantly with HtSDS ($r = -0.42$, $p < 0.001$). These data suggested that impaired liver functions, secondary to chronic viral hepatitis and/or siderosis, might contribute to linear growth impairment. Children with cirrhosis and chronic hepatitis are known to have a degree of GH resistance with low production of IGF-I (381,382).

Children with SCD did not have high prevalence of HBS antigenaemia or HCV antibody positivity. However, they had mild elevation of circulating ALT and bilirubin concentrations, which might be secondary to siderosis or hypoxic-ischaemic injury to the liver. ALT concentrations were correlated significantly with HtSDS in these children ($r=-0.21$, $p= 0.026$). SCD children with HBS antigenaemia had significantly elevated bilirubin and ALT concentration compared to those without antigenaemia. Two third of children with SCD who had acute hepatitis B infection could not clear the antigenaemia for one year after the attack . These data denoted that children with SCD are at high risk of developing chronic hepatitis following acute hepatitis B infection. Impaired hepatic function, which is more marked in those with HBS antigenaemia, might be a factor contributing to their linear growth delay.

This study demonstrated that impaired growth is associated with functional abnormalities of the growth hormone (GH)/ insulin-like growth factor -I (IGF-I)/ IGF-binding protein-3 (IGFBP3) axis as well as anatomical abnormalities of the pituitary

gland and its stalk in 21 short children with SCD. Nine out of the 21 children with SCD had defective GH response to both clonidine and glucagon provocation (peak <10 ug/L). These children differed from the other 12 in having slower linear growth velocity (GV and GVSDS), lower circulating concentrations of IGF-I and IGFBP3, and having either partial or complete empty sella. It appears that defective GH release and consequently low IGF-I production and slow growth velocity in children with SCD might be secondary to hypoxic-vascular insults to their hypothalamic-pituitary axis during one or more of the sickling episodes. In this group of patients with SCD defective GH secretion and consequently low IGF-I production are major etiologic factors causing their slow growth. The two groups with SCD, with and without GHD, did not differ significantly in their dietary intake, BMI, mid-arm circumference, skin-fold thickness, serum albumin concentration or intestinal absorption of D-xylose. These findings excluded a significant role played by malnutrition in the production of delayed growth in these patients.

A single injection of GH produced a smaller increase in circulating IGF-I in children with SCD with or without defective GH secretion compared to that in 10 age-matched children with idiopathic short stature (ISS) and 11 children with isolated GH deficiency (GHD). Moreover, human GH therapy (15units/m2/week, on daily divided subcutaneous doses) was started in two prepubertal children with SCD and empty sellae who had annual growth velocity (GV) of 3.2 and 3.5 cm/yr. respectively. Their GV increased to 9.7 and 8.1 cm/yr. on the first year of therapy and declined markedly to 5 and 4.1 cm/yr. and 3.6 and 3.9 cm/yr. on the second and third years of GH therapy respectively. Collectively; these findings denote a degree of resistance to GH in these patients. The presence of defective GH secretion, decreased IGF-I synthesis and partial resistance to GH in short children with SCD suggest that treatment with IGF-I may be superior to GH therapy for improving growth.

The presence of high incidence of empty sellae in short children with SCD stimulated us to compare their growth, biochemical, hormonal and radiological data with 3 other groups of non-sickler children with short stature. The incidence of empty sella was 45% in children with isolated GH deficiency, 80% in those with multiple pituitary deficiency, 100% in children with SCD and GH deficiency and 10% in the short children with normal hypothalamic-pituitary function. None of the children with normal stature had empty sella. These findings suggest that empty sella has a considerably high incidence in children with growth and hypothalamic pituitary disorders, particularly those with multiple pituitary hormone deficiency . This implicates the importance of CT scanning of the hypothalamic-pituitary area, in children with severe short stature including those with SCD, as an important tool in the identification of children with pituitary hormonal deficiency .
None of our patients with primary empty sella nor those with SCD and empty sella had diabetes insipidus. It seems that the presence of intrasellar CSF does not have an adverse effect on posterior lobe function. None of the children with empty sella had symptoms or signs of increased intracranial pressure, obesity or glucose intolerance reported in adults with empty sella (302).

Despite defective GH/IGF-I/IGFBB3 axis in children with SCD, their circulating 8. A.M. cortisol, free thyroxin (FT4), thyroid stimulating hormone (TSH) concentrations were normal. Cortisol response to ACTH stimulation was adequate in all of them. Their hormonal profile, apart from GH/IGF-I/IGFBP3 abnormalities, was not different compared to normal short children with constitutional growth delay (CSS) (n= 15). None of the children had impaired glucose tolerance.

Hypertransfusion therapy has dramatically increased the duration and quality of life in patients with thalassaemia major, however it leads to chronic iron overload, and is frequently complicated by the development of diabetes mellitus or impaired glucose tolerance and growth impairment. To determine the early effect of iron overload on the endocrine pancreatic function, we studied glucose, insulin and glucagon responses to oral load of glucose and to arginine provocation in 15 children with B-thalassaemia major, before and after (3.1+/-0.6 years) high-transfusion and iron chelation and compared them with 15 age matched normal controls. In addition we evaluated growth hormone (GH) responses to oral clonidine and measured the circulating insulin-like growth factor-I concentration in thalassaemic children on long-term transfusion and controls. After long-term high-transfusion, thalassaemic children had significantly decreased serum insulin concentrations and low insulin/glucose ratios at 60 and 120 minutes after an oral glucose load (1.75g/kg) in comparison with values before therapy and those for controls. None of the thalassaemic children had glucose intolerance after this period of frequent blood transfusion, however their serum glucose levels at 60 and 120 minutes after the oral glucose load were significantly higher compared to control children. Thirty minutes after starting arginine infusion, serum insulin concentration was significantly lower in thalassaemic children after versus before therapy. Basal and arginine-stimulated glucagon secretions were significantly elevated in thalassaemic children on long-term blood transfusion with significantly low serum insulin/glucagon ratios. In addition, the high basal serum glucagon concentrations were not suppressed after the oral glucose load. Despite hyperglucagonaemia in all thalassaemic children, their blood glucose dropped appropriately below 50% of the fasting glucose level after an intravenous insulin dose (0.1U/kg) ruling out significant insulin-resistance. GH responses to clonidine provocation were subnormal in thalassaemic children after long term blood transfusion compared to controls. In summary, thalassaemic children on long-term blood transfusion and iron chelation have progressive and early loss of B-cell mass, manifested by decreased insulin release in response to secretagogues, before the development of significant insulin resistance or impairment of glucose tolerance.

In vitro, cytokines like interleukin-1-beta (IL-1-B) and tumour necrosis factor-alpha (TNF-A) inhibit insulin release and can destroy islet B-cells. We measured blood levels of IL-1-B, TNF-A and islet cell antibody (ICA) in 20 children with IDDM, 20 of their non-diabetic siblings, 20 children with thalassaemia major on long-term hypertransfusion therapy and iron chelation, and 10 normal age-matched children. In the non-diabetic and thalassaemic children we investigated the early phase of insulin release after iv. glucose (0.5 g/kg, 30% solution) and evaluated tolerance to oral glucose (1.75 g/kg). Circulating IL-1-B and TNF-A concentrations were significantly higher in IDDM-siblings (33.7 +/- 12.7 pg/ml and 655 +/- 165 pg/ml respectively) versus normal children (21.1 +/- 6.4 pg/ml and 383 +/- 122 pg/ml respectively). Thalassaemic children had no detectable circulating ICA. The prevalence of ICA was 30 per cent in children with IDDM, and 60 per cent of their siblings. Impaired oral glucose tolerance was detected in 5 children with thalassaemia (25 per cent), but in none of the IDDM-siblings. The early phase of insulin release was significantly depressed in thalassaemic children (peak insulin = 29.2 +/- 5.1 mIU/ml) versus normal children (52.3 +/- 9.5 mIU/ml) and IDDM-siblings (45.3 +/- 12.4 mIU/ml). These data confirm the significantly decreased insulin secretion and impaired glucose tolerance in children with thalassaemia however, the mechanism of B-cell dysfunction is not mediated by neither ICA nor by cytokines.

The growth retardation of children with thalassaemia major is multifactorial. We studied the growth hormone (GH) response to provocation by clonidine and glucagon, measured the circulating concentrations of insulin, insulin-like growth factor-I (IGF-I),

IGF-binding protein-3 (IGFBP3), and ferritin, and evaluated the IGF-I generation after a single dose of GH (0.1 mg/kg/dose) in 15 prepubertal patients with thalassaemia and 15 age-matched children with constitutional short stature (CSS) (height SDS below -2, with normal GH response to provocation). Children with thalassaemia had significantly lower peak GH response to provocation by clonidine and glucagon (6.9 +/- 2.9 ug/L and 7.4 +/- 2.2 ug/L respectively) versus controls (18.6 +/- 2.7 ug/L and 16.7 +/- 3.7 ug/L respectively). They had significantly decreased circulating concentrations of IGF-I and IGFBP3 (49.6 +/- 21 ng/ml and 1.2 +/- 0.25 mg/L respectively) compared to controls (153 +/- 42 ng/ml and 2.06 +/- 0.37 mg/L respectively). These data document defective GH/IGF-I/IGFBP3 axis in thalassaemic children. Serum ferritin concentration was correlated significantly with GH peak response to provocation (r=-0.34, P<0.05), and circulating IGF-I (r = -0.45, P <0.01), and IGFBP3 (r = - 0.42, p< 0.01) concentrations. IGF−I generation test showed that after GH injection thalassaemic children have significantly lower IGF-I and IGFBP3 levels (90.8 +/- 10.8 ng/ml and 2 +/- 0.46 mg/L respectively) compared to controls (226 +/- 45.4 ng/ml, and 2.8 +/- 0.43 respectively). Six short (height SDS < -2) thalassaemic children who had defective GH response to provocation (< 10 ug/L) and 8 short normal children (CSS) were treated for one year with human GH (18 units/m2/week divided on daily S.C. doses). After one year of GH therapy there was a marked acceleration of growth velocity of both thalassaemic children (from 3.8 +/- 0.6 cm/yr. to 7.2 +/- 0.8 cm/yr.) and controls (9.9 +/- 1.2 cm/yr.). However, the linear growth velocity on GH therapy was significantly slower in thalassaemic children (3.3 +/- 0.3 cm increment) vs controls (5.3 +/- 0.4 cm increment) (p <0.05). After one year of GH therapy their circulating IGF-I concentrations (105 +/- 36 ng/ml) were significantly lower compared to controls (246 +/- 58 ng/ml). These data show that some children with thalassaemia major have defective GH/IGF-I/IGFBP3 axis and suggest the presence of partial resistance to GH.

We evaluated the spontaneous nocturnal (12-h) GH secretion and GH response to provocation in another group of prepubertal patients with thalassaemia and age-matched children with constitutional short stature (CSS) (height SDS < -2, with normal GH response to provocation). The anatomy of the hypothalamic pituitary area was studied in patients with abnormal GH secretion using MRI scanning. This piece of study confirmed that children with thalassaemia had significantly lower peak GH response to provocation by clonidine and glucagon (8.8 +/- 2.3 m/L and 8.2 +/- 3.1 mg/L respectively) versus controls (17.6 +/- 2.7 ug/L and 15.7 +/- 3.7 ug/L respectively). They had significantly decreased circulating concentrations of IGF-I and IGFBP3 (68.5 +/- 19 ng/ml and 1.22 +/- 0.27 mg/L respectively) compared to controls (153 +/- 42 ng/ml and 2.16 +/- 0.37 mg/L respectively). Seven of the thalassaemic children had GH peak response < 7mg/L after provocation. Thalassaemic patients with normal GH response after provocation, also had significantly lower IGF-I and IGFBP3 concentrations compared to controls. Serum ferritin concentration was correlated significantly with the circulating IGF-I (r = -0.47, P <0.01), and IGFBP3 (r = - 0.43, p< 0.01) concentrations.
Analysis of their GH pulse properties revealed lower mean nocturnal GH (2.9 +/- 1.77mg/L) and integrated nocturnal GH (2.53 +/- 1.6 mg/L) concentrations versus controls (4.9 +/- 0.29 mg/L and 5.6 +/- 0.52 mg/L respectively). They also had lower mean pulse amplitude (9.2 +/- 2.2 μg/L) versus controls (17.2 +/- 2 μg/L). Five of them had mean nocturnal GH concentration < 2mg/L and four had maximum nocturnal peak below 10 μg/L. These data denoted neurosecretory dysfunction of GH secretion in some of these patients.

MRI studies revealed complete empty sella (n= 2) , marked diminution of the pituitary size (n=4) , abnormal signal pattern of the gland (n=4) and thinning of the pituitary stalk (n=3) with its posterior displacement (n=2) in those patients with defective GH secretion (n= 9). Collectively, these data confirm the presence of high incidence of functional and structural abnormalities of the GH/IGF-I/IGFBP3 axis in short children with thalassaemia major as manifested by either defective GH response to provocation and/or neurosecretory dysfunction of GH secretion.

Patients with beta-thalassaemia major frequently have bone disorders of multifactorial aetiology. We attempted to analyse the relationship between bone mineral density (BMD) (measured by dual photon absorptiometry) on the one hand and auxologic parameters, degree of siderosis, function of the growth hormone (GH)/insulin-like growth factor-I (IGF-I)/ IGF-binding protein-3 (IGFBP3) axis, calcium-phosphate balance, parathormone (PTH), and cytokines {interleukin-1-beta (IL-1-B) and tumour-necrosis factor-alpha (TNF-A)} in 30 prepubertal children with thalassaemia major and 15 age-matched children with constitutional short stature (CSS), who have normal glucose tolerance and thyroid function. Children with thalassaemia had significantly decreased BMD and BMD% of average for age and sex (0.75 +/- 0.24 g/cm2 and 71 +/- 10% respectively) versus children with CSS (1.06 +/- 0.3 g/cm2 and 92 +/- 7% respectively). Thalassaemic patients had significantly lower circulating concentrations of IGF-I and IGFBP3 (49 +/- 21 ng/ml and 1.2 +/- 0.25 mg/L respectively) compared to control children (153 +/- 42 ng/ml and 2.1 +/- 0.37 mg/L respectively). GH response to provocation by clonidine and glucagon was defective (peak GH < 7 ug/L) in 12 out of the 30 thalassaemic children. Serum concentration of IL-1-B and TNF-A did not differ among the two study groups. hypocalcaemia was detected in 5 out of the 30 thalassaemic patients. Hypoparathyroidism was diagnosed in two of the 5 and rickets (osteomalacia) in the other 3 patients. The BMD was highly correlated with the circulating concentrations of IGF-I, IGFBP3 as well as with the auxologic parameters (age, weight, height, height standard deviation score and body mass index). It is suggested that increasing the circulating IGF-I concentration through aggressive nutritional therapy and/or via GH/IGF-I therapy with supplementation with vitamin-D and/or calcium might improve bone growth and mineralisation and prevent the development of osteoporosis and consequently fractures in these patients. Such therapy requires blinded controlled trials.

Patients with SCD also frequently have bone disorders of multifactorial aetiology. This study analysed the relationships between BMD on the one hand and auxologic parameters, degree of siderosis, function of GH/IGF-I/IGFBP3 axis, and calcium-phosphate balance in 28 prepubertal children with SCD and 15 age-matched children with constitutional delay of growth (CSS). Children with SCD had significantly decreased BMD (77.9 +/- 11.9 per cent of normal BMD for age and sex) and circulating concentrations of IGF-I (91 +/- 31 ng/ml) and IGFBP3 (1.7 +/- 0.44 mg/ml) versus the control group (BMD = 93.5 +/- 8.2 per cent of normal BMD for age and sex, IGF-I = 221 +/- 48 ng/ml, and IGFBP3 = 2.3 +/- 0.34 mg/ml). GH response to provocation was defective (peak below 10 ug/L) in 40 per cent of children with SCD. Sicklers with defective GH secretion had significantly lower circulating IGF-I concentration and BMD compared to those with normal GH secretion. Serum calcium, phosphate and alkaline phosphatase concentrations were normal in all children with SCD. The BMD was correlated significantly with height, weight, and body mass index as well as with the circulating concentrations of IGF-I and IGFBP3. It is suggested that increasing the circulating IGF-I concentration either through improving nutrition and increasing caloric intake and/or via GH/IGF-I therapy can improve growth and bone mineralisation in these patients.

In conclusion, children with thalassaemia major and SCD have significantly high incidence of delayed growth and sexual maturation which appear to be multifactorial. Assessment of the auxological, biochemical, hormonal and radiological data of these children has revealed many original facts and clarified others. 1. Many short thalassaemic and sickler children have defective GH secretion in response to provocation. 2. Some children with thalassaemia have neurosecretory dysfunction of GH secretion. 3. Both thalassaemic and sickler children have significantly decreased circulating IGF-I and IGFBP3 concentrations as well as defective IGF-I synthesis in response to exogenous GH injection suggesting a state of partial GH resistance (insensitivity). 4. Thalassaemic children on high-transfusion and iron chelation develop progressive insulin deficiency and hyperglucagonaemia before developing significant insulin-resistance or impaired glucose tolerance. Deficiencies of these growth factors (GH, IGF-I, IGFBP3, and insulin) appear to play a major role in the production of delayed growth in these patients. 5. Hepatic dysfunction, either due to siderosis and/or chronic viral hepatitis, might cause state of GH and insulin-resistance in thalassaemic patients.. 7. These functional abnormalities of the GH/IGF-I/IGFBP3 axis is accompanied by structural abnormalities of the pituitary gland and its stalk in patients with thalassaemia and SCD. 8. Thalassaemic children, but not those with SCD, might have other endocrinopathies including hypothyroidism, hypoparathyroidism and defective cortisol secretion in response to ACTH. 9. GH therapy produced increased growth rate in children with thalassaemia however, the increments of GV were significantly lower than for those with CSS or GHD denoting that these patients need supraphysiologic doses of GH to overcome their partial GH resistance. 10. Delayed and/or failure of puberty in patients with thalassaemia and SCD contributes to the aetiology of defective linear growth due to the failure or the attenuated pubertal (sex-steroid- dependent) growth spurt. 11. Children with thalassaemia and SCD have low BMD and are at high risk of developing osteoporosis and fractures.

ORIGINAL FINDINGS

1. Thalassaemia:

- **Forty nine percent of children with beta thalassaemia on frequent blood transfusion and iron chelation are short (HtSDS below -2) and eighty three percent of them had slow linear growth velocity (GVSDS below -1).**
- **Seventy three percent of thalassaemic males above the age of 14 years do not have testicular development. Spontaneous menarche and breast development occur only in 26 and 42 percent of thalassaemic females above the age of 13 years.**
- **Children with thalassaemia have high prevalence of HBS antigenaemia and HCV antibody seropositivity (44.4 and 23.5 percent) compared to normal age matched children. Serum ALT concentrations are significantly correlated with HtSDS. These findings suggest that impaired hepatic function secondary to siderosis or chronic hepatitis might contribute to linear growth impairment.**
- **Thalassaemic children have progressive and early dysfunction of pancreatic B-cells manifested by decreased insulin release in response to oral and i.v. glucose and arginine infusion, before the development of significant insulin resistance or impairment of glucose tolerance. They have significant hyperglucagonaemia not suppressible with oral glucose. The mechanism of B-cell dysfunction is not mediated neither by ICA nor by cytokines.**
- **Thalassaemic children have high prevalence of defective GH/IGF-I/BP3 axis as shown in this thesis by: 1. Defective GH response to provocation by clonidine and glucagon, 2. Defective spontaneous nocturnal secretion of GH (neurosecretory dysfunction of GH) in many children who have normal GH response to provocation, 3. Decreased circulating concentrations of IGF-I and IGFBP3, 4. Impaired IGF-I generation after exogenous injection of GH, 5. Slower linear growth velocity on GH therapy compared to children with CSS and those with GHD. The latter two findings suggest the presence of partial GH resistance in these patients.**
- **MRI scanning of their hypothalamic-pituitary area showed many structural abnormalities including different degrees of pituitary atrophy and haemosiderin infiltration of the pituitary gland and midbrain. These structural alterations are correlated with defective spontaneous GH secretion in these patients.**
- **Thalassaemic children had significantly reduced bone mineral density (BMD) which significantly increase the risk for fractures. Their BMD is correlated significantly with HtSDS and circulating concentrations of IGF-I and IGFBP3 and negatively with serum ferritin concentration. Hypocalcaemia in these patients might be secondary to hypoparathyroidism or rickets.**

2. SCD:

- **Twenty seven percent of children with are short (HtSDS below -2) and sixty seven percent of them had slow linear growth velocity (GVSDS below -1).**
- **Twenty two percent of males with SCD above the age of 14 years do not have testicular development. Spontaneous menarche occurs only in 50 percent of females with SCD above the age of 13 years.**
- **Children with SCD do not have high prevalence of HBS antigenaemia and HCV antibody seropositivity compared to normal age matched children. Sicklers with HBS antigenaemia have higher serum ALT concentration versus those without the antigenaemia. Serum ALT concentrations are significantly correlated with HtSDS. These findings suggest that impaired hepatic function secondary to siderosis or chronic hepatitis might contribute to linear growth impairment.**
- **Children with SCD have high prevalence of defective GH/IGF-I/BP3 axis as shown in this thesis by: 1. Defective GH response to provocation by clonidine and glucagon, 2. Decreased circulating concentrations of IGF-I and IGFBP3, 3. Impaired IGF-I generation after exogenous injection of GH, 4. Rapid deceleration of linear growth after the first year of GH therapy.**
- **CT scanning of the hypothalamic-pituitary area of sicklers with defective GH secretion reveals empty sella in 100 percent of these patients suggesting anoxic injury of the pituitary gland during one or more of the sickling episodes with secondary degeneration and atrophy.**
- **Children with SCD had significantly reduced bone mineral density (BMD) which significantly increase the risk for fractures. Their BMD is correlated significantly with height, weight , and BMI as well as with the circulating concentrations of IGF-I and IGFBP3 and negatively with serum ferritin concentration.**

RECOMMENDATIONS

1. **High- and or super- transfusion and iron chelation programmes should be optimised to offer adequate haemoglobin level without the deleterious effects of iron overload on all tissues including the liver, pancreas, and pituitary gland in patients with transfusion -dependent thalassaemia and SCD.**
2. **Aggressive nutritional support to improve the quality and quantity of food intake in these patients should be an integral part of their treatment protocol.**
3. **Vaccination against hepatitis B and meticulous screening of blood before transfusion should help reduce the high prevalence of chronic hepatitis and deterioration of hepatic function in these patients.**
4. **Linear growth should be monitored closely in these patients and short children (HtSDS below -2) or those with slow GV should be investigated for GH/IGF-I deficiency. In addition, thyroid function and glucose tolerance of short and/or slowly growing children with thalassaemia should be investigated.**
5. **Adolescents with delayed and/or failure of puberty should be investigated early for gonadotropin release and sex-steroid secretion. Those with hypogonadism should be treated early and adequately with either gonadotrophins (human chorionic gonadotropin) and or sex-steroids to improve their pubertal growth spurt, bone accretion and sexual development and function.**
6. **Studying the anatomy of the pituitary gland by MRI imaging is a helpful tool to prove the structural abnormality and support the finding of defective GH secretion in these children.**
7. **Children with thalassaemia and SCD who have defective GH secretion and low circulating IGF-I should receive either GH and/or IGF-I replacement therapy to improve linear growth and increase bone mass. However, patients with thalassaemia might need supraphysiologic doses of GH to overcome their partial GH resistance.**
8. **A large double-blind controlled study is required to investigate the long-term effect of GH/IGF-I replacement, vitamin-D and calcium supplementation, and gonadotropin/ sex-steroid replacement on the bone accretion and bone mass density in these patients.**

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APPENDIX

FOREWORD

Thalassaemia and sickle cell disease (SCD) are the most widely distributed blood genetic disorders that occur at a high frequency in some populations including the Mediterranean region, parts of the Middle East, South East Asia and the Indian subcontinent. It is estimated that thalassaemia major affects 100,000 new-born every year world-wide. The high incidence of these chronic haemolytic diseases in developing countries poses a high load on the national economy because of the expensive treatment protocols and the considerably high morbidity rates of these patients. Repeated blood transfusion to keep haemoglobin above an acceptable level requires well-equipped blood banks with expensive facilities to screen, store and manipulate blood and blood products. Iron chelation therapy is an essential part of treatment to avoid or delay the deleterious effects of iron overload on different organs including the liver, heart, pancreas and endocrine glands. This requires injecting deferoxamine subcutaneously for 12 hours daily with a special pump. Both deferoxamine and pumps are expensive and therefore not accessible for all patients.

In developing countries, the majority of transfusion-dependent patients with chronic haemolytic anaemia (thalassaemia and SCD) suffer from the consequences of sub-optimal treatment. The mortality rate is still high and usually patients die before the age of 30 years. They also suffer from chronic multi-organ damage including cardiac failure, liver cirrhosis, insulin-dependent diabetes mellitus, growth and pubertal failure and many skeletal abnormalities and fractures. In developed countries the introduction of high transfusion regimes and efficient chelation therapy improved survival rates and prevented cardiac and hepatic damage. However, a majority of thalassaemic patients still have significant growth and pubertal abnormalities, bone disease and multiple endocrine disorders.

In Egypt the incidence of thalassaemia major ranges between 0.1 - 0.2% which gives very high patient load on the medical services. In our University of Alexandria Children's Hospital, Alexandria, Egypt. The Haematology clinic has an average of 150 thalassaemic children registered. The same problem is encountered by me in the Royal Hospital, Muscat, Oman, with high prevalence of SCD and thalassaemia and suboptimal treatment. Because of the restricted economic resources, both hospitals adopt a low transfusion therapy (to keep haemoglobin above 9 g/dl) with IM chelation 3 times per week. With this form of sub-optimal treatment we observed that a large number of our thalassaemic children have severe growth and pubertal failure/delay, beside other hepatic, cardiac and skeletal abnormalities. In fact they constitute 40% of patients attending our Endocrinology clinic. This stimulated me to perform an extensive study to survey growth and pubertal development in theses patients (study-1) and investigate the different factors that might affect their growth and pubertal development (studies 4 through 10) as well as bone mass density (studies 11,12). The frequent involvement of the liver in these patients led us to study some hepatic functions and the prevalence of transfusion-associated hepatitis B surface antigenaemia and hepatitis-C virus antibody seropositivity in relation to

their linear growth (studies 2,3). We studied the nutritional intake of these patients, their intestinal absorption of D-Xylose and 48-h stool fat content in relation to their body mass index, subcutaneous fat thickness and mid-arm circumference (studies 4,5,9).

Their defective linear growth urged us to investigate their growth hormone (GH) secretion (spontaneous nocturnal as well as after provocation) and insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 (IGFBP3) concentrations. Our findings demonstrated high prevalence of defective GH secretion in these children that necessitated imaging of their hypothalamic pituitary area. Imaging studies revealed original data about structural abnormalities in the anterior pituitary gland, different degrees of pituitary atrophy and empty sella and infiltration the gland as well as the mid-brain by haemosidrin in thalassaemic children, the mechanism of these findings was explained (studies 4-6, 10). Because of their slow growth, the presence of abnormal GH/IGF-I/BP3 axis, and structural abnormalities of the pituitary gland, the next step dealt with the response of IGF-I to exogenous GH and the clinical response of their linear growth to GH therapy for a year or more (studies 4,9).

Based on the fact that these patients have high prevalence of bone pains and osteoporosis during late childhood and have high risk of spontaneous fracture thereafter, we measured their bone mass density to investigate the relation between the former and the degree of iron load, growth parameters, and different anabolic hormone concentrations in these patients (studies 11,12).

The overview contains the details of all the 12 studies, summary and recommendations. For tables and figures please refer to the original papers (1-12) included in the APPENDIX.

Growth and Pubertal Development in Transfusion-Dependent Children and Adolescents with Thalassaemia Major and Sickle Cell Disease (SCD): A Comparative Study

by Ashraf T. Soliman MD,* Mahmoud ElZalabany MD,* Maysa Amer MD, *and* B. M. Ansari FRCP**

**Department of Paediatrics, University of Alexandria Children's Hospital, Alexandria, Egypt*

***Department of Paediatrics, East Glamorgan General Hospital, Church Village, and School of Applied Sciences, University of Glamorgan, Pontypridd, Wales, UK*

Summary

Despite regular blood transfusion and desferrioxamine treatment, growth impairment and pubertal delay are commonly seen in children and adolescents with transfusion-dependent thalassaemia and sickle cell disease (SCD). We evaluated growth parameters and sexual maturation in a large cohort of children and adolescents with SCD ($n = 110$) and thalassaemia ($n = 72$) receiving nearly the same protocol of transfusion and chelation, and compared them with those for 200 normal age-matched children, 30 children with constitutional delay of growth (CSS), and 25 children with growth hormone deficiency (GHD). Before transfusion, haemoglobin concentration had not been less than 9 g/dl in the past 7 years; desferrioxamine was administered for 7–10 years, including by the intramuscular and subcutaneous routes, three times or more per week. The height standard deviation score (HtSDS), growth velocity (GV) (cm/yr), and growth velocity standard deviation score (GVDSD) of children and adolescents with thalassaemia and SCD were significantly decreased compared to normal children ($p < 0.01$). Forty nine per cent of thalassaemic patients and 27 per cent of patients with SCD had HtSDS less than −2, and 83 per cent of thalassaemic patients and 67 per cent of SCD patients had HtSDS less than −1. Fifty six per cent of thalassaemic children and 51 per cent of children with SCD had GVSDS less than −1. The GV of thalassaemic children was significantly slower than that for children with SCD. Children with thalassaemia and SCD had HtSDS and GVSDS comparable to those for children with CSS but higher than those for patients with GHD. Serum ferritin concentration was correlated significantly with the linear GV in all patients ($r = 0.45$, $p < 0.001$). The bone age delay did not differ among the three groups with thalassaemia, SCD and CSS, but the delay was significant in the group with GHD. The mid-arm circumference was significantly smaller in children with thalassaemia and SCD than in normal children. The triceps skin-fold thickness of patients with SCD was significantly decreased compared to thalassaemic and normal children. The upper/lower segment ratio was significantly lower in thalassaemic and SCD patients than in normal children. In thalassaemic patients between the ages of 13 and 21 years a complete lack of pubescent changes was present in 73 per cent of boys and 42 per cent of girls. Seventy four per cent of the thalassaemic girls had primary amenorrhoea. Girls with SCD aged between 13 and 21 years had markedly delayed breast development and menarche. Twenty five per cent of boys with SCD above the age of 14 years had absence of testicular development. Males with thalassaemia and SCD who had spontaneous testicular development had significantly smaller testicular volume than did normal controls. Short children with thalassaemia and SCD had significantly decreased serum insulin-like growth factor 1 (IGF-1) concentrations compared to children with CSS. Collectively, these data confirm the high prevalence of impaired growth and pubertal delay/failure in children and adolescents with thalassaemia and SCD. The aetiology of impaired growth includes the contributions of lack of pubertal growth spurt due to delayed/absent puberty, decreased synthesis of IGF-1 which might be secondary to a disturbed GH–IGF-1 axis and/or under nutrition, probably due to the hypermetabolic status of these children. It is suggested that newer protocols of treatment, in addition to optimization of transfusion and chelation requirements, should increase the caloric intake of these patients and properly manage their pubertal delay-failure in order to improve their adult height.

Correspondence: Ashraf T. Soliman MD, Associate Professor of Pediatrics, University of Alexandria Children's Hospital, 3 Abdel Sattar Mansour Street, Loran, Alexandria, Egypt. Tel. 00 20 2 543 9076; Fax 00 20 3 587 6828.

Journal of Tropical Pediatrics 648 JTP JT0195 AP 15/9/98 15:31 ALDEN

Introduction

The prognosis for transfusion-dependent chronic haemolytic anaemias has greatly improved.[1-22] With more intensive transfusion regimens and administration of adequate chelation therapy, life expectancy has increased. Growth and pubertal delay and/or failure represent major obstacles to the fulfilment of successful therapy of these diseases.[8,9] Controversy still exists about the effect of different regimens of blood transfusion and chelation on linear growth and pubertal development in these patients. Some investigators claim normalization of growth and pubertal development[10,11] whereas others report only suboptimal improvement of linear growth.[6-9] We performed a systematic survey of growth and pubertal development in a large cohort of patients with thalassaemia major and sickle cell disease (SCD), all of whom had received the same treatment during the past 10 years, to investigate the effect of this regimen on growth and pubertal maturation of these patients.

Patients and Methods

All patients with transfusion-dependent thalassaemia major and SCD were randomly selected from the Haematology Clinics of the Royal Hospital and Alexandria University Children's Hospital, Alexandria, Egypt. The participating hospitals were chosen with consideration of homogeneity of the treatment protocol. Mean pre-transfusion haemoglobin (Hb) concentration was 9 g/dl in the past 7 years in most patients; individual variability was not such as to permit comparisons between patients. They received desferrioxamine (50 mg/kg/dose) by intramuscular or subcutaneous injection three times weekly. All were on folic acid supplements and all had been vaccinated against pneumococci. Two hundred age- and sex-matched normal, randomly selected children, 30 children with constitutional delay of growth (CSS) (HtSDS ≤ -2, with delayed bone age and normal growth hormone (GH) response to provocation), and 25 children with isolated GH deficiency (GHD) served as controls. None of the children had a history of intrauterine growth retardation, any other systemic or endocrine disease, dysmorphic trait, or central nervous system irradiation. Informed consent for the testing procedures was obtained from the parents of all children. All children were examined with special emphasis on nutritional data. A special form was prepared for this study. The anthropometric measurements included weight to the nearest 100 g, height to the nearest mm, mid-arm circumference (MAC), and triceps and subscapular skin-fold thickness during a hospital visit at the time of the study, Harpenden's callipers and anthropometric measurements were used. The data recorded were the average of three sequential measurements determined by the same observer (A.S.T.). The height standard deviation score (HtSDS) was calculated according to the formula $\text{HtSDS} = (X_1 - X_2)/\text{SD}$, where X_2 and SD are the age-matched population mean height and SD, respectively, and X_1 is the subject's height. The height growth velocity (GV), in cm/year, was calculated for a complete year, following the first measurement. Normal population data were according to Tanner *et al.*[23] The body mass index (BMI) was calculated according to the formula weight (kg)/[height (m)]^2. The bone age and stages of sexual maturation were evaluated according to Greulich and Pyle[24] and Tanner *et al.*[23] respectively, and the age of menarche and early breast development were recorded.

In a randomly selected sample of short children (height less than fifth centile for age and sex) from each group [thalassaemia ($n = 15$), SCD ($n = 21$), and CSS ($n = 10$)] a fasting venous blood sample was obtained and kept frozen at −20°C until analysed for thyroxine (free T_4, FT_4), thyrotrophin (thyroid-stimulating hormone, TSH), and insulin-like growth factor 1 (IGF-1) concentrations by radioimmunoassay. All samples from all children were assayed simultaneously. IGF-1 concentrations were measured by radioimmunoassay, employing reagents purchased from the Nichols Institute (San Juan Capistrano, CA, USA). The intra-assay coefficient of variation averaged 8.2 per cent in the range of IGF-1 concentrations measured. Free T_4 and TSH were measured using Amerlex-RIA kits (Kodak Clinical Diagnostics). Statistical analyses were performed using the ANOVA test to compare mean analyte concentrations among the different groups. Statistical significance was accepted at $p < 0.05$. The linear regression equation was used to test the relation between variables.

Results

The anthropometric and bone age data of the four study groups are presented in Table 1. The HtSDS, GV, and GVSDS of patients with thalassaemia and SCD were significantly decreased compared to normal children ($p < 0.01$). The HtSDS of thalassaemic children was significantly lower than that for children with SCD. Both groups had HtSDS higher than those with GHD. The GV and HVSDS of patients with thalassaemia were lower than that for SCD, but the difference did not attain statistical significance for the GVSDS ($p = 0.09$). Children with thalassaemia and SCD had GV and GVSDS significantly higher that those with GHD but not different from those with CSS. The bone age delay (years) did not differ among the three study groups with thalassaemia, SCD, and CSS. The BMI of thalassaemic patients was significantly higher than that for the other groups. The means of the mid-arm circumference and triceps skin-fold thickness were significantly decreased in children with SCD compared to normal children (Table 2). In thalassaemic children, the mid-arm circumference was significantly smaller than that for normal controls. Their triceps skin-fold thickness was comparable to that for normal children. Assessment of the food intake, using the recall method for the past three

Journal of Tropical Pediatrics 648 JTP JT0195 AP 15/9/98 15:31 ALDEN

TABLE 1
Anthropometric and bone age data (mean ± SD)

	Bone age delay (years)	BMI (kg/m^2)	GVSDS	GV (cm/year)	HtSDS	Age (years)
Thalassaemia ($n = 70$)	1.9	16.7*†	−1.2*	3.15*†	−2*†	10.8
	0.8	2.5	0.7	1.63	1.2	2.9
SCD ($n = 110$)	2.4	14.6	−1.02*	4.99*	−1.4*	7.2
	1.2	1.3	1.07	2.1	0.86	2.4
CSS ($n = 30$)	2.25	14.1	−1.1*	4.6*	−2.1*	10.4
	1	0.4	0.5	0.04	0.45	1.8
GHD ($n = 25$)	3.1	15.2	−2.9*	2.8*	−3.5*	8.6
	1	2	1	1	0.4	2.9
Normal	ND	15.8	0.21	6.2	0.39	10.5
		0.45	0.05	0.16	0.12	3.4

* $p < 0.05$, groups with disease vs. normal children.
† $p < 0.05$, thalassaemia vs. SCD.

days, failed to detect any qualitative or quantitative deficiency in food consumption by children with thalassaemia and SCD. The upper segment/lower segment (U/L) ratio in children with thalassaemia (0.85 ± 0.07) and SCD (0.89 ± 0.09) was significantly lower than for age-matched controls (1.09 ± 0.6).

Figures 1 and 2 and Table 3 show the HtSDS, GVSDS, and BMI data of children with thalassaemia and SCD. Linear growth was significantly impaired in both groups of children with thalassaemia and SCD. Forty nine per cent of the thalassaemic children and 27 per cent of those with SCD had HtSDS < −2. Eighty three per cent of thalassaemic children and 67 per cent of children with SCD had HtSDS below −1. A considerably large percentage of thalassaemic and sickle-cell children had slow growth velocity; 56 per cent of thalassaemic children and 51 per cent of children with SCD had GVSDS below −1 during a full year of linear growth. Figure 3 shows scattergrams of chronological age versus HtSDS and GVSDS in patients with thalassaemia and SCD. The age was correlated negatively with HtSDS ($r = 0.405$, $P < 0.01$) and GVSDS ($r = 0.2$, $p = 0.04$) (Fig. 3) suggesting progressive growth retardation with

TABLE 2
Mid-arm circumference (MAC) and triceps skinfold thickness (Tri-SFT)

	MAC (cm)	Tri-SFT (mm)
Thalassaemia ($n = 72$)	16.5*	9.5
	2.4	2.5
SCD ($n = 10$)	14.3*†	8.02*†
	1.3	1.5
Normal ($n = 200$)	19.8	9.2
	1.8	1

* $p < 0.05$, groups with disease vs. controls.
† $p < 0.05$, thalassaemia vs. SCD.

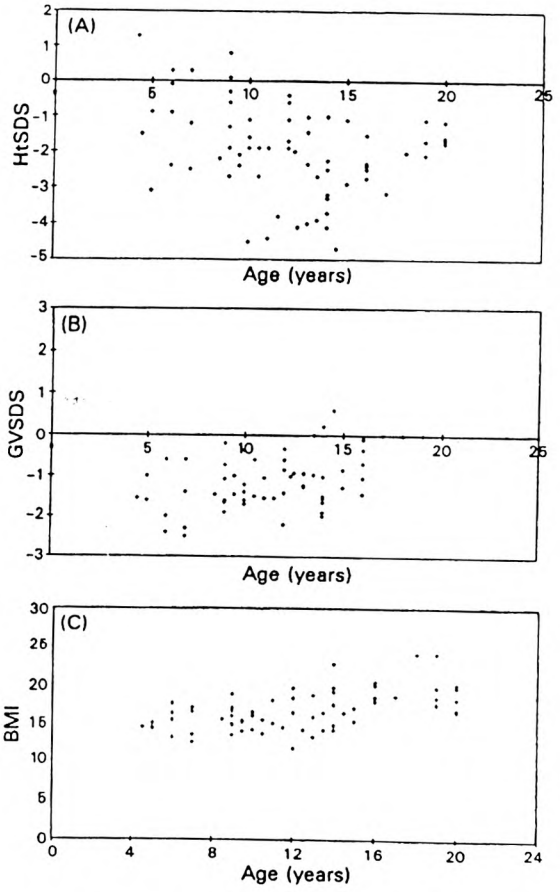

FIG. 1. Growth data for patients with thalassaemia major. (a) HtSDS vs. age; (b) GVSDS vs. age; and (c) BMI vs. age.

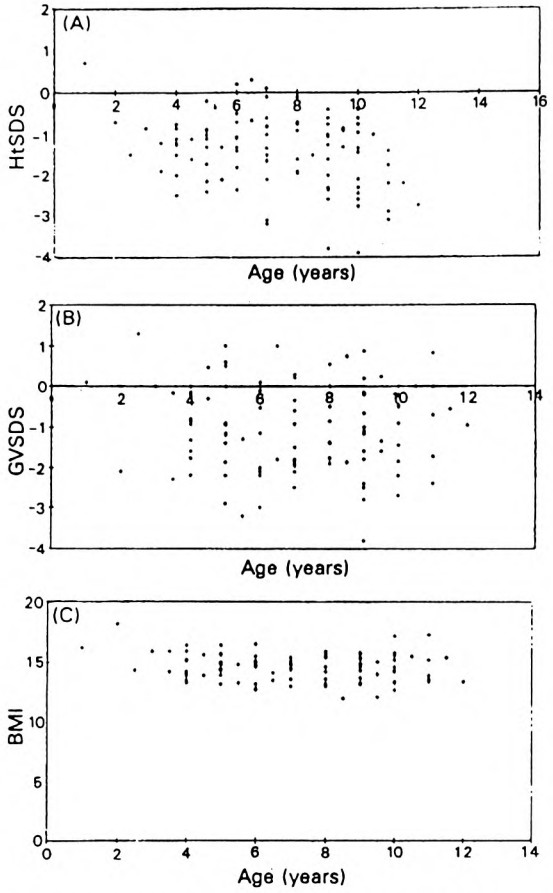

FIG. 2. Growth data for patients with SCD. (a) HtSDS vs. age; (b) GVSDS vs. age; (c) BMI vs. age.

age in these patients. Serum ferritin concentration was correlated negatively with GV ($r = 0.45$, $p < 0.001$) (Fig. 4).

Pubertal development data are shown in Table 4. If testicular volume ≥3 ml and breast development >B1 are taken as evidence of the beginning of pubertal development, a considerable number of patients had not experienced sexual maturation, even at ages when this is the rule in normal subjects. The data from 22 thalassaemic boys between the ages of 14 and 21 years (16.9 ± 3.8 years) showed that only six (27 per cent) of them had testicular enlargement (volume >3 ml). Their mean testicular volume was 6.8 ± 2.5 ml. Out of the 19 thalassaemic girls between the ages of 13 and 22 years (17.2 ± 3.2 years) only five (26 per cent) had spontaneous menarche at a mean age of 17.5 ± 1.2 years. Breast development was delayed in 11 (B2 at mean age of 15.7 ± 1.5 years) and absent in eight of them. Three out of the five patients who had spontaneous menarche had irregular menstrual cycles and two of the three had secondary amenorrhoea.

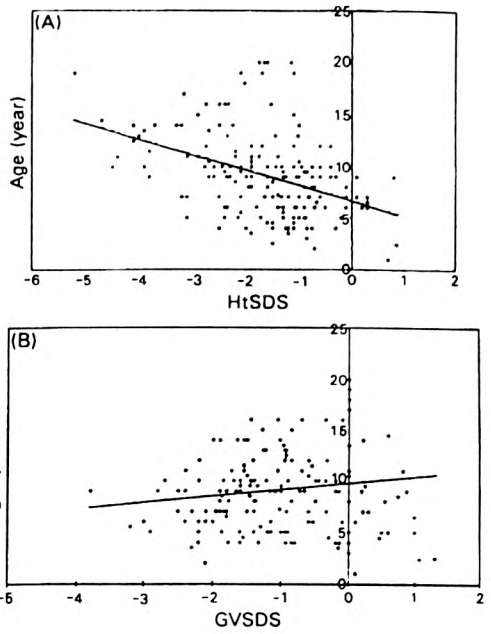

Fig. 3. Relationship between age and (a) HtSDS ($r = -0.405$, $p < 0.001$) and (b) GVSDS ($r = -0.02$, $p = 0.04$).

In girls with SCD above the age of 13 years (16.5 ± 3.2) ($n = 16$), 14 girls had delayed breast development (B2) after the age of 13 years (mean age of 13.5 ± 0.4 years) and only two had breast development before the age of 13 years (B2 at mean age of 12.5 ± 0.4 years). Seven girls out of the 14 had spontaneous menarche at an age of 15.6 ± 0.7 years. Out of the 18 boys with SCD above the age of 14 years (17.1 ± 2.8 years) had testicular volume above 3 ml (Tanner score of 2 or more) and four (25 per cent) had testicular volume <3 ml at the age of 14, 14.5, 15.5, and 16 years respectively. Their mean testicular volume was 8.5 ± 3.1 ml. The pubertal status of 30 normal males (age 16.9 ± 2.6 years) and 30 normal females (age

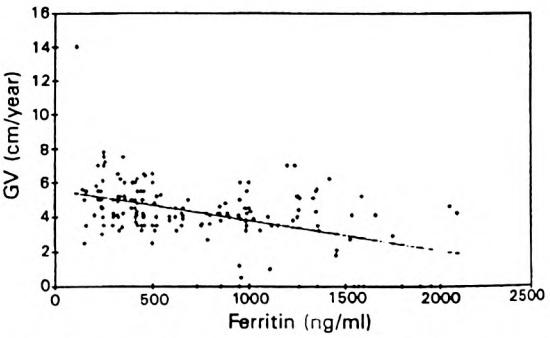

Fig. 4. Relationship between serum ferritin level and growth velocity ($r = -0.45$, $p < 0.001$).

TABLE 3
Height and growth velocity SDS in patients with thalassaemia and SCD

HtSDS	%	*n*	GVSDS	%	*n*
SCD					
< -2	27	31	< -2	19	21
$> -2 < -1$	40	44	$> -2 < -1$	32	35
$> -1 < 0$	28*	30	$> -1 < 0$	27	29
> 0	5	5	≥ 0	22	24
Total	100	110		100	110
Thalassaemia					
< -2	49*	35	< -2	10	7
$> -2 < -1$	35	25	$> -2 < -1$	46	74
$> -1 < 0$	9	7	$> -1 < 0$	24	17
≥ 0	7	5	≥ 0	19	14
	100	72		100	72

*$p < 0.05$ between groups.

TABLE 4
Pubertal development in patients and normal controls

	Sex	*n*	Age (years)	B2 (years)	Menarche (years)	Testicular volume (ml)
Thalassaemia	M	22	16.9 (3.5)	–	–	6.8 (2.5)*
	F	19	17.2 (3.2)	15.7 (1.5)*	17.5 (3.1)*	–
SCD	M	16	17.1 (2.8)	–	–	8.5 (3.1)*
	F	18	16.5 (3.2)	13.? (1.2)*	15.6 (0.7)*	–
Controls	M	30	16.2 (2.3)	10.7 (0.8)	–	12.4 (3.4)
	F	30	16.9 (2.6)	–	13.4 (1.2)	–

Menarche: age at first menstrual period; B2: age at 'tanner stage 2' of breast development.
*$p < 0.05$, groups vs. controls.

16.2 ± 2.3 years) between the ages of 13 and 21 served as controls. The mean age for starting breast development (B2) ranged between 9.5 and 13.1 years with a mean of 10.7 ± 0.8 years. The mean age of menarche was 13.4 ± 1.2 years. All the normal adolescent boys ($n = 30$, age 16.9 ± 2.6 years) had testicular volume above 4 ml. Their mean testicular volume was 12.4 ± 3.4 ml.

The auxological, biochemical, and hormonal data of the short children (height less than fifth percentile) with thalassaemia, SCD, and CSS are presented in Table 5. Serum ferritin and alanine-transferase concentrations were significantly higher in thalassaemic children versus the other two groups. Insulin-like growth factor 1 concentration was significantly lower in children with thalassaemia and SCD than in children with CSS. Circulating IGF-1 concentrations were significantly lower in children with thalassaemia than in those with SCD. FT_4 and TSH concentrations did not differ among the three study groups.

Discussion

Subjects from the study cohort were diagnosed either at birth or during infancy and constitute a representative sample of children with thalassaemia and SCD. Analysis of cross-sectional and longitudinally collected auxological data and comparison with normal children as well as with two groups of children with CSS and isolated GHD allowed better evaluation of growth in these patients. The present data show the high prevalence of short stature in children with thalassaemia and SCD during childhood and adolescence. This was more marked in thalassaemia; about half of the thalassaemic children had HtSDS < -2, denoting marked linear growth retardation. In addition the linear growth velocity of more than half of the children with thalassaemia and SCD was slow (GVSDS < -1). Again this slow GV was more marked in thalassaemic children than in children with SCD. The HtSDS and GVSDS of children with thalassaemia and SCD were comparable to those for children with CSS and significantly higher than for children with GHD. The skeletal age, as well, was more delayed in children with GHD versus those with thalassaemia and SCD. Collectively these data, in accord with the results of other investigators,[1–9] confirm a delay in linear growth in children with thalassaemia and SCD. The significant (negative) correlation between the chronological age on the one hand and the HtSDS and GVSDS on the other hand point to the fact that linear growth delay increases progressively with age in these patients.

Journal of Tropical Pediatrics 648 JTP JT0195 AP 15/9/98 15:31 ALDEN

TABLE 5
Auxological parameters and biochemical data

		CSS ($n = 10$)	SCD ($n = 21$)	Thalassaemia ($n = 15$)
M/F		5/5	12/9	8/7
Age (years)	Mean (SD)	8.1 (0.8)	7 (3.3)	6.9 (2.8)
B one age delay (years)	Mean (SD)	−2.5 (0.8)	−2.1 (1)	−1.5 (0.45)
HtSDS1	Mean (SD)	−1.9 (0.3)	−2.1 (0.6)	−2.2 (0.5)
HtSDS2	Mean (SD)	−2.1 (0.5)	−2.2 (0.55)	−2.4 (0.6)
GV (cm/years)	Mean (SD)	4.6 (1.2)	4.3 (1.2)	3.8 (0.43)
BMI (kg/m^2)	Mean (SD)	15.1 (0.6)	14.5 (1.8)	15.8 (1.15)
Ferritin (ng/ml)	Mean (SD)	99 (54)	521** (446)	1595** (328)
ALT U/L ratio	Mean (SD)	18.7 (11.5)	35 (7.8)	70.2* (35)
Bilirubin (μmol/l)	Mean (SD)	5.8 (4.5)	23* (12)	21* (5.4)
Albumin (g/l)	Mean (SD)	39.4 (6.2)	43.8 (4.1)	41.2 (5.2)
IGF-1 (ng/ml)	Mean (SD)	168 (43)	109* (68)	82.4* (24)
FT_4	Mean (SD)	17.5 (2.5)	16.1 (1.5)	15.8 (1.5)
TSH (mIU/ml)	Mean (SD)	1.5 (0.5)	1.4 (0.4)	2.9 (0.77)

* $p < 0.05$, groups vs. CSS.

The mean MAC of patients with thalassaemia and SCD was significantly smaller than that for the age-matched controls. In addition, children with SCD had significantly smaller skin-fold thickness and lower BMI than normal controls. These findings indirectly denoted decreased mis-arm muscle mass in patients with thalassaemia and SCD and decreased subcutaneous fat deposition in patients with SCD. Undernutrition, either due to decreased food intake and/or secondary to a hypermetabolic state[25,26] due to increased bone marrow activity, and/or deficiency of the anabolic hormones (e.g. IGF-1) could be aetiologic factors. The higher BMI in patients with thalassaemia does not accurately reflect the nutritional status because of the frequent finding of considerable hepatomegaly and/or splenomegaly in thalassaemic patients, disturbing the ratio of weight and height. Assessment of dietary intake of these patients did not detect any qualitative or quantitative abnormality, denoting that probably other factors such as hypermetabolism and/or defective intestinal absorption of nutrients[27] might be the case.

Delayed onset of puberty was a frequent finding in both boys (testicular enlargement) and girls (breast develoment and menarche) with thalassaemia and SCD. The delay/failure of puberty was more pronounced in patinets with thalassaemia, with more than 70 per cent of girls having primary or secondary amenorrhea and more than 70 per cent of boys older than 14 years having delayed testicular development. In these patients the significantly low U/L segment ratio might reflect the hypogonadal status of these children, but it might also be due to vertebral changes secondary to hyperactivity of the bone marrow.

The aetiology of retarded growth in children with thalassaemia and SCD is probably multifactorial, with contributions from abnormal endocrine function,[8–11,20,22,28–34] suboptimal nutrition,[34,35] an increase in metabolism due to hyperactivity of the bone marrow,[25,26] and hypogonadism.[8,9] In this study, serum IGF-1 concentrations were significantly depressed in short children with thalassaemia and SCD compared to short normal children, denoting defective IGF-1 synthesis in these patients. This might be due to defective GH secretion[36–40] and/or resistance to GH due to liver siderosis.[41] The significant (negative) correlation between GV (cm/year) and serum ferritin concentrations supports the view that decelerated growth occurs with increasing iron load. Undernutrition, shown in our patients by reduced mid-arm circumference and subcutaneous fat thickness, might contribute to growth delay in these children through decreased IGF-1 synthesis. Although analysis of qualitative and quantitative food intake, using the recall method, failed to detect any abnormality, undernutrition might nevertheless be caused by the hypermetabolic state of these children presumably due to hyperactivity of their erythropoiesis. Some investigators report improvement of growth after increasing the caloric intake of these patients.[42] Moreover, in many hypermetabolic states there is some degree of GH resistance, which might be a contributing factor in decreased synthesis of IGF-1 and consequently delayed growth.[43] Although other endocrinopathies, like hypothyroidism and diabetes mellitus, could adversely affect growth in these patients, none of the patients included in this study had abnormal thyroid function or impaired glucose tolerance.

Delayed and/or failure of puberty occurred in considerably large numbers of males and females with thalassaemia and SCD. The lack of the synergistic action of sex steroids on the pubertal growth spurt appears to be a major factor contributing to growth delay in these patients. In this study, HtSDS and GVSDS were correlated negatively with chronological age, denoting that as patients with thalassaemia grow older without achieving pubertal development, their linear growth becomes more delayed. The low U/L segment ratio in

patients, similar to those with hypogonadism, supports the concept that lack of sex steroid affects linear growth in these patients.

Collectively, the marked delay or failure of growth and puberty in children with thalassaemia major and SCD denotes that treatment with the current transfusion and chelation protocol is suboptimal and necessitates the application of a more aggressive programme of hypertransfusion and iron chelation to improve growth in these patients. Improvement of nutritional status, by increasing caloric intake, should be an essential part of any new protocol of treatment for these patients. Early detection of delayed/failed puberty and management by proper physiological replacement of sex steroids and/or gonadotrophins should improve their pubertal growth.

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Journal of Tropical Pediatrics 648 JTP JT0195 AP 15/9/98 15:31 ALDEN

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Journal of Tropical Pediatrics 648 JTP JT0195 AP 15/9/98 15:31 ALDEN

tudy of Hepatic Functions and Prevalence of lepatitis-B Surface Antigenaemia in Omani Children ith Sickle Cell Disease

Ashraf T. Soliman,* MD, Mohamed R. Bassiouny,** MD, *and* Nagwa A. Elbanna,* MS
Department of Pediatrics, Royal Hospital, Muscat, Oman
Sultan Qaboos, University Hospital, Muscat, Oman

Summary

The prevalence of persistent hepatitis-B surface (HBS) antigenaemia and hepatic functions have been determined in 125 children with sickle cell disease (SCD) as well as in 100 age-matched healthy children. Hepatic functions and the presence of HBS antigenaemia have been followed for 1 year in six children with SCD and 10 normal children following acute hepatitis-B infection. The prevalence of chronic HBS antigenaemia (3 per cent) in children with SCD is not higher than in normal children (11 per cent). The significant elevation of serum alanine transferase (ALT) and bilirubin concentrations in sickle cell children denotes a process of mild hepatocellular dysfunction which is unrelated to hepatitis-B viral antigenaemia.

The high incidence of chronic HBS antigenaemia accompanied by elevated serum ALT and bilirubin concentrations in sickle cell children following acute hepatitis-B infection, in addition to the significant impairment of hepatic functions in sicklers with chronic HBS antigenaemia compared to those without the antigenaemia, point out to the high risk of continual parenchymal hepatic damage in these children following acute hepatitis-B infection. Vaccination against hepatitis-B virus should eliminate this risk.

Introduction

Jaundice can occur in any haemolytic anaemia, owing to an increase in the level of unconjugated bilirubin. Patients with chronic haemolytic anaemias, undergoing repeated blood transfusions, have an increased risk of developing hepatitis.[1] In sickle cell disease the liver is often enlarged and tender during acute crisis, with some abnormalities of hepatic functions.[2] Histology reveals clumps of erythrocytes, platelets, and fibrin in the sinusoids with area of focal necrosis.[2–4] Occasionally, the hepatic manifestations may dominate the clinical picture with conjugated hyperbilirubinaemia and bilirubinuria. These attacks closely mimic viral hepatitis. Although the clinical findings in patients with sickle hepatic crisis, who have hepatitis-B antigenaemia, are similar to the findings in other patients, the histological changes differ, with aggressive cellular infiltration of the portal tracts and disruption of the limiting plates.[5] One report raises the possibility that viral hepatitis might be more severe in these patients.[6]

In a large series of adults with sickle cell anaemia hepatic histology showed cirrhosis in 10 per cent of them, with unexplained hepatic necrosis, portal fibrosis, and regenerative nodules.[7] In addition, Masera and colleagues reported high rate of occurrence of chronic hepatitis following acute hepatitis B and non-A non-B attacks in thalassemic patients.[8,9]

Correspondence: Ashraf T. Soliman, MD, Department of Pediatrics, Royal Hospital, Seeb 1331, Muscat, Oman.

This led us to study the prevalence of persistent hepatitis-B surface antigenaemia in children suffering from sickle cell disease and their significance in relation to hepatic functions.

Materials and Methods

One-hundred-and-twenty-five children between the ages of 3 and 12 years with sickle cell disease were the subject of this study. They represented the sickle cell children in Muscat area attending the Pediatric Hematology Clinic of the Royal Hospital, Muscat, Oman. One-hundred normal age-matched children served as controls. All the study children were subjected to thorough history taking, with special emphasis on previous episodes of jaundice, injections, and blood transfusions. Full clinical examination was performed, and the size of the liver and spleen recorded. Blood samples were collected for estimation of serum alanine transferase (ALT), alkaline phosphatase (ALP), albumin, and bilirubin concentrations. All serum samples of the study children were tested for the presence of hepatitis B surface antigen (HBSAg) using the latex quick test.[10]

Hepatic function tests and hepatitis-B surface antigenaemia have been followed every 3 months, for 1 year, in six sicklers and 10 normal children with acute hepatitis B infection.

Statistical analyses were done using the *t*-test for comparison between the different study groups when

the data were normally distributed and Wilcoxon test when they were not. Data are presented as mean ± SD.

Results

Table 1 shows the hepatic functions of children with SCD and controls. Four out of the 125 children with SCD were HBSAg carriers (3 per cent), while 11 out of the 100 normal children were carriers. Serum bilirubin and ALT concentrations were higher in the sickle group compared to the controls. Serum ALT and bilirubin levels were significantly higher in HBSAg negative sicklers ($n=121$) than those for HBSAg negative controls ($n=89$) (Table 2). Table 3 compares the liver functions of HBSAg positive sicklers and controls. Serum bilirubin and ALT concentrations were significantly higher and serum albumin levels were markedly lower in the sicklers' group. Table 4 presents a comparison between two groups of sickle cell children with and without HBS antigenaemia.

TABLE 1
Hepatic functions and HBS antigenaemia in children with sickle cell disease and controls

	Age (months)	Bili (μmol/l)	ALT (IU/l)	ALP (IU/l)	Albumin (g/l)	Hb (g/dl)	HBSAg positive
Children with SCD ($n=125$)	108 ± 4.4	38.6 ± 3.8*	41.2 ± 6.3*	141 ± 7.1	38.7 ± 3.0	8.3 ± 0.3*	3.2% (4)
Normal children ($n=100$)	90.0 ± 5.2	17.1 ± 2.8	21.9 ± 1.9	161 ± 9.2	37.4 ± 1.1	12.5 ± 0.8	11% (11)

*$P<0.05$. Bili = bilirubin.

TABLE 2
Hepatic functions and HBS antigenaemia in children with sickle cell disease and controls

	Age (months)	Bili (μmol/l)	ALT (IU/l)	ALP (IU/l)	Albumin (g/l)	Hb (g/dl)
Children with SCD ($n=121$)	108.6 ± 4.5	38.9 ± 3.9*	40.4 ± 6.3*	141 ± 7.3	38.9 ± 3.1	8.0 ± 0.2*
Normal children ($n=89$)	97.2 ± 5.1	17.9 ± 3.2	20.4 ± 1.7	164 ± 10.0	37.5 ± 1.3	12.5 ± 0.5

*$P<0.05$. Bili = bilirubin.

TABLE 3
Hepatic functions in HBSAg positive children with sickle cell disease and controls (mean ± SD)

	No	Age (months)	Bili (μmol/l)	ALT (IU/l)	ALP (IU/l)	Albumin (g/l)	Hb (g/dl)
Children with sickle cell disease	4	123 ± 12	35.5 ± 3.5*	90 ± 25*	131.5 ± 3.5	30.0 ± 6.0*	7.8 ± 0.5*
Normal children ($n=100$)	11	104 ± 16	12.0 ± 3.4	29.8 ± 8.0	144.9 ± 24.2	37.0 ± 2.7	12.8 ± 1.0

*$P<0.05$.

TABLE 4
Hepatic functions in sickle children with and without surface antigenaemia (mean ± SD)

	No	Bili (μmol/l)	ALT (IU/l)	ALP (IU/l)	Albumin (g/l)	Hb (g/dl)
HDSAg positive sicklers	4	35.5 ± 3.5	90 ± 25*	131.5 ± 3.5	30.0 ± 6.0*	7.8 ± 0.5*
HBSAg negative sicklers	121	38.9 ± 3.9	40.4 ± 6.3	142.7 ± 7.3	38.9 ± 3.1	8.0 ± 0.2

*$P<0.05$.

tive children. In all the five study groups ALT concentrations were significantly higher in HCV seropositive compared to seronegative children. Liver size was significantly larger in HCV seropositive control children, as well as those with thalassemia, and SHF *v.* children who were seronegative in each group, respectively.

Discussion

Hepatitis-C virus can be detected in blood within 7–14 days of exposure and persists throughout the course of infection. However, the presence of circulating HCV antibody cannot be confirmed until 9–20 weeks after exposure. This creates a window of seronegativity and potential infectivity.[2] Acute infection carries a high risk of developing chronic hepatitis and liver cirrhosis. The HCV antibody test is used to detect acute and chronic HCV infections. It is positive in approximately 50 per cent of patients with acute HCV infection and 80–90 per cent with chronic NANB hepatitis.[2,15,16]

In this study the prevalence of HCV antibody in normal ($n = 110$) Egyptian children is 12 per cent. This is a markedly higher prevalence rate compared to those reported from different African and Asian countries.[11–14] It appears that the risk and magnitude of HCV infection are relatively high in Egypt.

The major factors in community-acquired HCV infection include blood transfusions, intravenous drug abuse, and inapparent percutaneous exposure.[15] One study has suggested that household and sexual transmission of HCV is most inefficient.[17] In three of the high risk groups of children we studied, namely those with thalassemia major, IDDM, and SHF, the prevalence of HCV antibody positivity has been significantly high (44, 29, and 38 per cent, respectively). This confirms that parental transmission, either through intravenous or subcutaneous route, is a major factor in community-acquired infections. In addition, this high prevalence of HCV seropositivity in these groups of children poses a considerable danger for development of chronic liver disease. The presence of significantly larger liver size in HCV seropositive normal children as well as HCV seropositive children with thalassemia and SHF, and the significant elevation of their serum ALT concentrations compared to HCV seronegative children in each group, respectively, strongly support this view. Moreover, Masera and colleagues has reported high rate of occurrence of chronic liver disease following acute NANB-hepatitis in thalassemic patients.[18,19]

In conclusion, the prevalence of HCV antibody seropositivity is relatively high (12 per cent) in a random sample ($n = 110$) of healthy Egyptian children and significantly high in children with thalassemia major (44 per cent), SHF (38 per cent), and IDDM (29 per cent).

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Sicklers with HBS antigenaemia had significantly higher ALT and lower albumin concentrations compared to those without HBS antigenaemia.

Out of six children with sickle cell disease who presented with HBSAg positive acute hepatitis, two patients did not clear the antigen from their serum after one year, with elevated serum bilirubin (59 and 85 umol/l) and ALT (87 and 110 IU/l) concentrations. None of the normal children with acute HBSAg positive hepatitis were still carrying the antigen after 3 months of the attack.

Discussion

This study shows that children with sickle cell disease do not have a high prevalence of HBS antigenemia. Our results confirm the findings from Nigeria that the incidence of hepatitis in sickle cell children is no higher than the incidence in the general population.[11]

Significant elevation of serum ALT and bilirubin concentrations in children with sickle cell disease compared to control children denotes the presence of mild chronic hepatocellular dysfunction which might be due to repeated vascular-hypoxic insults and/or chronic viral inflammation. However, this is not related to hepatitis-B virus because it is present also in sickle cell patients without HBS antigenaemia.

Previously normal persons usually clear the antigen from the serum within 4–6 weeks from the onset of acute symptoms. Chronic hepatitis is associated with persistent antigenemia.[12] In our study, 33 per cent of sicklers with acute hepatitis-B infection did not clear the antigenaemia for 1 year whereas, all the normal children cleared the antigen from the serum after 3 months. In addition, the comparison in Table 4 proves that in children with sickle cell disease chronic carrying of HSAg is accompanied with more deterioration of hepatic functions (high ALT and bilirubin concentrations). These findings point to the high risk of chronicity and continual parenchymal damage in sicklers after acute hepatitis-B infections. This can drastically influence the prognosis of hepatic involvement in this disease. It appears that vaccination against hepatitis-B should be a useful tool to eliminate this risk.

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Prevalence of Hepatitis-C Antibody Seropositivity in Healthy Egyptian Children and Four High Risk Groups.

by Ahmed A. El-Nanawy,* MD, Omar F. El Azzouni,* MD, Ashraf T. Soliman,* MD, A.E. Amer,** MD, R.S. Demian,*** MD, *and* H.M. El-Sayed,*** MD

**Department of Pediatrics, Universities of Alexandria, Egypt*

***Department of Pediatrics, University of Zazazig, Egypt*

****Medical Research Institute, Alexandria, Egypt*

Summary

We studied the prevalence of HCV antibody seropositivity and serum alanine concentrations in a random sample of healthy Egyptian children ($n=110$) as well as in four high risk groups of children. Group 1 included 18 children with thalassemia major, group 2 included 17 children with insulin-dependent diabetes mellitus (IDDM), group 3 included 21 children with schistosomal hepatic fibrosis (SHF), and group 4 included 20 children with chronic rheumatic heart disease (RHD).

The prevalence rate of HCV seropositivity was 12 per cent in normal children, 44 per cent in thalassemic children, 29 per cent in children with IDDM, 38 per cent in children with SHF and 0 per cent in patients with RHD. The liver size was significantly larger in HCV seropositive normal children as well as in HCV seropositive children with thalassemia and SHF compared to the seronegative children in each group respectively ($P<0.05$). In all groups serum alanine transferase concentrations were significantly higher in HCV seropositive *v.* seronegative children. This pointed out to the high risk of continuous parenchymal hepatic damage in these children following acute HCV infection.

In summary, our data revealed a relatively high prevalence of HCV antibody seropositivity in healthy Egyptian children compared to reports from other countries, and a significantly high prevalence of HCV seropositivity in children with thalassemia, IDDM, and SHF which carries a considerably high risk for development of chronic liver disease in these patients.

Introduction

Hepatitis-C virus (HCV) has a global distribution and causes parentally acquired and sporadic non-A-non-B (NANB) hepatitis. It is responsible for the majority of post-transfusion NANB hepatitis.[1–3] The non-parental modes of spread are poorly defined. Intrafamilial transmission suggests a horizontal rather than a vertical mode of spread.[4–7] In addition, HCV has been incriminated in the aetiology of chronic liver disease and hepatic malignancy.[8–10]

The prevalence of HCV antibody varies among different populations. It ranges between 0.3 and 1.5 per cent in some Western countries and Taiwan[2,8,11] to 6 per cent in Zaire.[12] In a population based survey in Saudi Arabia significant variations in the prevalence of HCV antibody are reported from different regions with a range from 0 to 6 per cent.[13,14]

The aim of this study was to estimate the prevalence of HCV antibody in a sample of healthy Egyptian children ($n=110$) as well as in four high risk groups of children with schistosomal hepatic fibrosis, thalassemia, insulin-dependent diabetes mellitus (IDDM), and chronic rheumatic heart disease (RHD).

Materials and Methods

One-hundred children between the age of 1 and 16 years were the subject of this study. Group 1 included 18 children with thalassemia major on regular blood transfusion and iron chelation therapy, group 2 included 17 children with insulin-dependent diabetes mellitus (IDDM), group 3 included 21 children with schistosomal hepatic fibrosis (SHF) and group 4 included 20 children with chronic rheumatic heart disease (RHD). All were randomly selected from the Hepatology, Diabetology, Hematology and Cardiology out-patient clinics of Alexandria University Children's Hospital, Alexandria, Egypt. One-hundred-and-ten healthy age-matched children served as a control group. All the study children were subjected to thorough history taking, with special emphasis on previous episodes of jaundice, injections, and blood transfusions. Informed consent for the blood testing was obtained from the parents of all children before including in the study. Full clinical examination was performed and the size of the liver recorded. Blood samples were collected for estimation of serum alanine transferase (ALT) concentrations. All

serum samples of the study children were tested for the presence of IgG antibody to HCV by indirect ELISA test (Behring).

Analysis of variance (ANOVA) was used to test the differences between mean values in the study groups. Significance was accepted at $P<0.05$. Data are presented as mean ± SD.

Results

Table 1 presents the prevalence of HCV antibody, liver size and ALT concentrations in the five study groups. Children with SHF were significantly older than children in the other groups. The prevalence of HCV seropositivity was significantly higher in three of the high risk groups (thalassemia, IDDM, and SHF) compared to the control group. In the RHD group the prevalence of HCV antibody was 0 per cent. Liver size was significantly increased on palpation in the thalassemic and SHF groups compared to the other groups. ALT concentrations were significantly higher in the thalassemic and SHF groups *v.* the control group.

Table 2 compares ALT concentrations and liver size among the HCV antibody seropositive and seronega-

TABLE 1
Prevalence of HCV antibody, liver size, and ALT concentration in the five study groups

Groups		Age (years)	Male (%)	Female (%)	Positive *n* (%)	Liver (cm)	ALT (IU/l)
Normal control	Mean	8.6	44.5	55.5	13 (11.8)	5.7	28.2
$n=110$	SD	4.3				1.2	5.5
	range	(1–16)				(3–8.1)	(10–80)
Thalassemic	Mean	11.4	44.5	55.5	8 (44.4)*	10.1*	53.9*
$n=18$	SD	3.9				1.6	6.9
	range	(6–16)				(8.2–13)	(45–65)
IDDM	Mean	9.6	82.3	17.7	5 (29.4)*	7	39.3
$n=17$	SD	4				0.7	10.9
	range	(4.5–15)				(6–7.8)	(30–55)
SHF	Mean	13.6	76.2	23.8	8 (38.1)*	8.3*	41.4*
$n=21$	SD	1.8				0.66	9.3
	range	(10–15)				(7–9.7)	(10–35)
RHD	Mean	10.5	75	25	0	6	24.3
$n=20$	SD	3.2				0.7	7.3
	range	(3.5–16)				(4–7.3)	(10–35)

*$P<0.05$ groups *v.* control. Positive = HCV antibody seropositive.

TABLE 2
Liver size and ALT concentration in seropositive v. *seronegative children*

Groups		ALT (IU/l) positive	ALT (IU/l) negative	Liver (cm) positive	Liver (cm) negative
Normal	Mean	60	24*	7.1	5.5*
	SD	13.4	9.8	0.8	1
Thalassemic	Mean	58.8	50*	8.2	10.2
	SD	4.8	6.1	0.7	1.8
IDDM	Mean	55	33*	7.55	6.8
	SD	2.3	1.8	0.35	0.7
SHF	Mean	56.3	32.3*	9	7.8*
	SD	3.5	7.8	0.4	0.3
RHD	Mean	0	24.3	0	6
	SD	0	7.3	0	0.7
Total	Mean	58.5	32.6*	7.9	6.6*
	SD	6.8	6.2	0.6	0.8

*$P<0.05$ seropositive *v.* seronegative.

tive children. In all the five study groups ALT concentrations were significantly higher in HCV seropositive compared to seronegative children. Liver size was significantly larger in HCV seropositive control children, as well as those with thalassemia, and SHF *v.* children who were seronegative in each group, respectively.

Discussion

Hepatitis-C virus can be detected in blood within 7–14 days of exposure and persists throughout the course of infection. However, the presence of circulating HCV antibody cannot be confirmed until 9–20 weeks after exposure. This creates a window of seronegativity and potential infectivity.[2] Acute infection carries a high risk of developing chronic hepatitis and liver cirrhosis. The HCV antibody test is used to detect acute and chronic HCV infections. It is positive in approximately 50 per cent of patients with acute HCV infection and 80–90 per cent with chronic NANB hepatitis.[2,15,16]

In this study the prevalence of HCV antibody in normal ($n = 110$) Egyptian children is 12 per cent. This is a markedly higher prevalence rate compared to those reported from different African and Asian countries.[11–14] It appears that the risk and magnitude of HCV infection are relatively high in Egypt.

The major factors in community-acquired HCV infection include blood transfusions, intravenous drug abuse, and inapparent percutaneous exposure.[15] One study has suggested that household and sexual transmission of HCV is most inefficient.[17] In three of the high risk groups of children we studied, namely those with thalassemia major, IDDM, and SHF, the prevalence of HCV antibody positivity has been significantly high (44, 29, and 38 per cent, respectively). This confirms that parental transmission, either through intravenous or subcutaneous route, is a major factor in community-acquired infections. In addition, this high prevalence of HCV seropositivity in these groups of children poses a considerable danger for development of chronic liver disease. The presence of significantly larger liver size in HCV seropositive normal children as well as HCV seropositive children with thalassemia and SHF, and the significant elevation of their serum ALT concentrations compared to HCV seronegative children in each group, respectively, strongly support this view. Moreover, Masera and colleagues has reported high rate of occurrence of chronic liver disease following acute NANB-hepatitis in thalassemic patients.[18,19]

In conclusion, the prevalence of HCV antibody seropositivity is relatively high (12 per cent) in a random sample ($n = 110$) of healthy Egyptian children and significantly high in children with thalassemia major (44 per cent), SHF (38 per cent), and IDDM (29 per cent).

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Circulating Growth Hormone (GH), Insulin-like Growth Factor-I (IGF-I) and Free Thyroxine, GH Response to Clonidine Provocation and CT Scanning of the Hypothalamic-pituitary Area in Children with Sickle Cell Disease

by Ashraf T. Soliman,* MD, Assim Darwish,** MD, Said H Mohammed,** MD, Mohammed R Bassiony,*** MD, Nagwa El Banna,* MD, *and* Maurice Asfour,† MD

*Departments of *Pediatrics, †Endocrinology and **Radiology, The Royal Hospital and Qaboos University, ***College of Medicine, Sultanate of Oman*

Summary

Serum growth hormone (GH), cortisol, free thyroxine (FT4), thyroid-stimulating hormone (TSH), and insulin like growth factor I (IGF-I) concentrations were measured in 15 children with sickle cell disease (SCD) together with their heights <5th percentile for age and gender, and in 15 healthy age-matched children who had normal variant short stature (NVSS). GH response to an oral dose of clonidine (0.15 mg/m^2) and cortisol response to ACTH stimulation were determined in the two groups.

Children with SCD had significantly lower serum concentrations of IGF-I and decreased GH response to stimulation. Eight out of the 15 children with SCD did not mount an appropriate GH response to clonidine provocation (>10 μg/l). CT scanning of the hypothalamic-pituitary area in those eight children with SCD revealed a partial or complete empty sella in all of them. It appears that defective GH release, and consequently low IGF-I production and slow growth velocity in children with SCD might be secondary to hypoxic-vascular insults to their hypothalamic-pituitary axis during one or more of the sickling episodes.

Introduction

Growth and maturational delay are striking features of sickle cell disease (SCD).[1] After 6 months of life, affected children have impaired growth involving both the height and weight.[2–4]

Many factors have been implicated in the aetiology of growth impairment in children with SCD. These include chronic anaemic hypoxia, increased energy expenditure due to high erythropoietic turn-over and cardiac work,[5] nutritional deficiencies including zinc, folic acid and vitamin-A,[6–8] disturbed calcium metabolism,[9] repeated infections due to defective immune functions,[10] and dysfunction of the endocrine glands.[12]

The basal circulating concentrations of different hormones have been studied by various authors,[12–14] with no consensus in defining the endocrine status and response of different glands to provocation in children with SCD, and their possible contribution to growth impairment in these children.

Our aim was to estimate the circulating levels of IGF-I, free thyroxine (FT4), thyroid-stimulating hormone (TSH), and the responses of GH and cortisol to provocation by clonidine and adrenocorticotrophic hormone (ACTH), respectively, in children with SCD who had short stature [height (Ht) <5th percentile] for age and gender. These hormonal data were analysed in relation to the anthropometric, clinical, and biochemical data of the patients.

Patients and Methods

Fifteen prepubertal children with SCD and short stature, height standard deviation score (HtSDS) < -2, attending the Pediatric Hematology/Endocrinology Clinic of the Royal Hospital, Muscat, Oman, were the subjects of this study. All children were on regular blood transfusion to keep their haemoglobin (Hb) concentrations above 9 g/dl. All were on folic-acid supplements and all had been vaccinated against pneumococci. Fifteen age-matched children with normal variant short stature (NVSS) served as controls. None of the children had history of intra-uterine growth retardation, any other systemic or endocrine disease, dysmorphic trait, or central

Correspondence: Dr Ashraf T. Soliman, Pediatric Endocrinology, Royal Hospital, PO Box 1331 Seeb, Muscat, Sultanate of Oman.

nervous system irradiation. Informed consent for the testing procedure was obtained from the parents of all children. All the children were examined with special emphasis on nutritional data. The anthropometric measurements included weight, height, and mid-arm circumference. Harpenden's calipers and anthropometric measurements were used. The data recorded were the average of three sequential measurements determined by the same observer (AST). The HtSDS was calculated according to the formula HtSDS = (XI − X2)/SD, where X2 and SD are age matched population mean height and SD, respectively, and XI is the subject height. The height growth velocity (GV) cm/year was calculated for the last year. Normal population data were according to the Tanner *et al.*[15] The body mass index (BMI) were calculated according to the formula = weight (kg)/height (m)^2. The bone age was determined according to Greulich and Pyle atlas.[16]

On the day of admission, venous blood samples were obtained for determination of complete blood count (CBC) and serum albumin, bilirubin, alanine transferase (ALT), alkaline phosphatase (ALP), calcium, phosphorus, and bicarbonate concentrations. Following an overnight fast (8-h) venous blood samples were withdrawn through a polyethylene catheter inserted in a forearm vein between 8 and 9 a.m. The serum was separated from the formed elements by centrifugation and kept frozen at −20°C until analysed for GH, FT4, TSH, and IGF-I. After obtaining the basal samples, an oral dose of clonidine was given (0.15 mg/m^2). Blood samples were obtained every 30 min for 2 h for measurement of serum GH concentrations. On the next morning and after an 8-h overnight fast 0.5 mg of ACTH (synacthen) was injected i.v. and blood samples obtained before and 60 min after the injection for estimation of cortisol concentrations. Human GH was measured by

TABLE 1

Anthropometric and bone age data of children with sickle cell disease (SCD) and those with normal variant short stature (NVSS)

	Age (years)	GV (cm/year)	Ht-SDS	BMI (kg/m^2)	Bone age delay (years)
SCD ($n=15$)	7.9 ± 1.1 5.7–11.5	5.0 ± 0.5 (3.4–6)	−2.15 ± 0.18 (−1.95– −3)	13.9 ± 0.53 (0.5–4)	2.25 ± 1.0 (1.5–4)
NVSS ($n=15$)	3.1 ± 0.5 (6–11)	4.6 ± 0.4 (3.9–5.9)	−2.65 ± 0.3 (−2– −3)	13.8 ± 0.5 (12–18.9)	2.9 ± 0.25 (0.5–3)

GV = growth velocity; Ht-SDS = height standard deviation score; BMI = body mass index; $P > 0.05$.

TABLE 2

Biochemical data of children with SCD and those with NVSS (mean ± SD)

	Allbumin (g/l)	ALT (IU/l)	Bilirubin (μmol/l)	Calcium (mmol/l)	Phosphorus (mmol/l)	ALP (IU/l)	Creatinine (ml/min/1.73m^2)	Bicarb (mmol/l)	HB (g/dl)	Hct
SCD ($n=15$)	39 ± 3 (32–44)	40 ± 28* (18–80)	37.3 ± 11* (14–43)	2.1 ± 0.08 (2–2.28)	1.3 ± 0.082 (1.2–1.4)	23 ± 92 (98–380)	132 ± 9.1 (70–158)	20.5 ± 2.6 (17–24)	8.5 ± 1.5* (8–11)	0.27 ± 0.06* (0.29–0.34)
NVSS ($n=15$)	37 ± 4 (36–45)	21 ± 13 (14–50)	15 ± 5 (8–9)	2.2 ± 0.1 (2.1–2.4)	1.4 ± 0.09 (1.3–1.66)	161 ± 25 (120–197)	120 ± 9.3 (91–145)	22.2 ± 1.8 (18–24)	12.8 ± 1.1 (10.5–5–14)	0.37 ± 0.04 (0.34–0.45)

ALT = alanine transferase; ALP = alkaline phosphatase; Hb = haemoglobulin; Hct = haematocrit. *$P < 0.05$.

TABLE 3

Hormonal data of patients with SCD and those with NVSS (mean ± SD)

	FT4 (pmol/l)	TSH (μIU/ml)	GH basal (μg/l)	GH peak (μg/l)	Cortisol basal (nmol/l)	Cortisol-peak (nmol/l)	IGF-I (IU/l)
SCD ($n=15$)	16.6 ± 0.87 (13.2–23.3)	1.8 ± 0.32 (0.7–2.7)	1.8 ± 0.25 (0.5–3.5)	9.2 ± 2.0 (1.5–25.6)	451 ± 43 (247–704)	841 ± 60 (513–1151)	9.8 ± 1.5 (3–19)
NVSS ($n=15$)	17.4 ± 0.6 (14.9–21.7)	1.6 ± 0.15 (1–2.4)	1.6 ± 0.22 (0.3–3.3)	19.6 ± 2.7 (10–31)	466 ± 62 (388–620)	1062 ± 208 (730–1390)	22.1 ± 4.8 (6–54)

radioimmunometric assay, employing reagents purchased from Nichols Institute (San Juan Capistrano, CA). Intra-assay coefficient of variations averaged 6 per cent in the range of GH values detected and 5.8 per cent in the range of IGF-I concentrations measured. All samples from all the children were assayed simultaneously.

CT examination of the hypothalamic pituitary area have been performed in all sicklers who did not mount an appropriate GH response to clonidine provocation ($n=8$). One of the children with SCD had a second CT performed after intrathecal injection of the contrast material.

Statistical analyses were done using the unpaired t-test to compare mean analyte concentrations among the two study groups when the data were normally distributed and Wilcoxon test when they were not. Statistical significance was accepted at $P<0.05$.

Results

The anthropometric and bone age data of the two study groups are presented in Table 1. The chronological age, HtSDS, body mass index (BMI), and bone age did not differ significantly between the two groups ($P>0.05$).

The biochemical data of all the children are presented in Table 2. Creatinine clearance and serum concentrations of creatinine, bicarbonate, albumin, and ALP were not different among the two groups. Haemoglobin concentrations and haematocrit values were lower in children with sickle cell disease and their serum ALT and bilirubin concentrations were higher compared to the control children.

The hormonal profiles of all the children are shown in Table 3. The circulating IGF-I concentrations and the peak GH levels in response to clonidine provocation were significantly lower in the sickle cell group. Eight out of the 15 children with sickle cell disease did not mount a proper GH peak >10 μg/l after clonidine intake, whereas all children with NVSS mounted normal GH response >10 μg/l. Basal and ACTH-stimulated cortisol levels as well as FT4 and TSH concentrations did not differ significantly between the two groups. CT scanning of the hypothalamic-pituitary area revealed a picture of complete ($n=5$) or partial ($n=3$) empty sella in all the eight children with

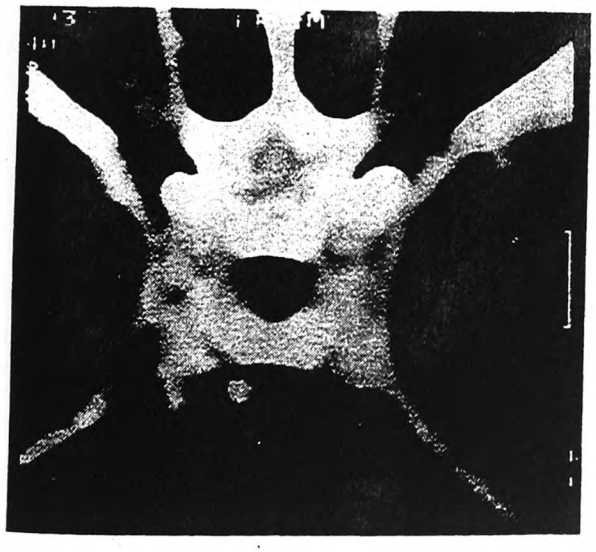

FIG. 1. CT of a 7-year-old boy with SCD and empty sella. Average attenuation values of the sella was 6 Hounsfield Units.

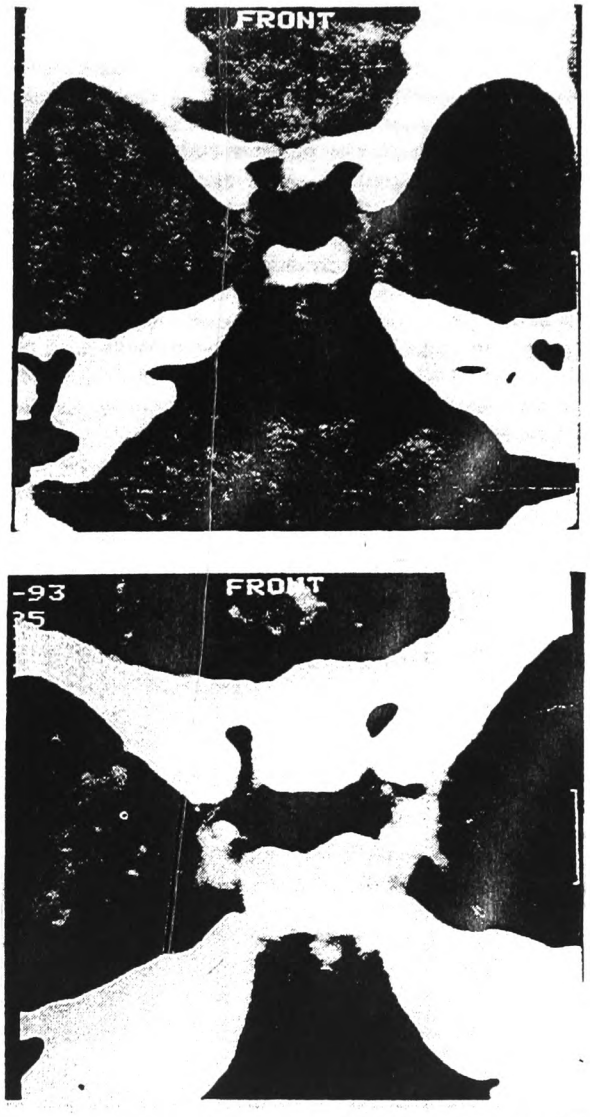

FIG. 2. CT of a 10-year-old girl with SCD: (a) CT without i.v. contrast medium; and (b) CT taken after i.v. injection of contrast medium. Attenuation values of the sella contents were between -35 and 8 Hounsfield Units; compatible with partial empty sella with fatty degenerative changes.

SCD who had defective GH response to clonidine. In those patients with complete empty sella the contents of the sella had densities around cerebrospinal fluid (CSF) values Fig. 1, whereas in those with partial empty sella the contents of the pituitary fossa were between −45 and −27 which was suggestive of some fatty degenerative changes Fig. 2. In the patient who had CT done after intrathecal injection of contrast medium Fig. 3 a significant herniation of the suprasellar cistern into the pituitary fossa was noted. Plain skull X-rays for the pituitary fossae were normal in all children with NVSS and SCD.

Discussion

In this study we compared the growth, biochemical, and hormonal data of two groups of children with sickle cell disease and normal variant short stature (NVSS). The hormonal profile of children with sickle cell disease revealed defective GH response to clonidine provocation as well as low circulating concentrations of IGF-I compared to the control group. Malnutrition can be excluded as a cause of these changes because the BMI and serum albumin concentrations were not different among the two study groups. In addition, in malnutrition the basal GH concentrations are usually high unlike the case in our children with sickle cell disease.[15] The normal creatinine clearance, and normal concentrations of serum creatinine, bicarbonate, calcium, phosphorus, and alkaline phosphatase exclude any contribution of renal dysfunction, and/or disturbed calcium homeostasis in the aetiology of growth impairment or hormonal abnormalities in these children.

Although serum ALT and total bilirubin concentrations were higher in the group with sickle cell disease compared to the control group, those ALT levels of sicklers were still in the normal range of population (10–60 IU/l) and the unconjugated bilirubin constituted the main part of the elevated bilirubin. This in addition to normal concentrations of serum albumin and ALP, and normal albumin/globulin ratio makes it unlikely that hepatocellular dysfunction is a cause for low IGF-I production in these patients.

It appears that in children with sickle cell disease low GH secretion leads to decreased synthesis of IGF-I and, consequently, to slow growth velocity and short stature. This defective GH release might be secondary to a hypoxic-vascular insult to the hypothalamic pituitary area and/or an intrinsic abnormality in the children associated with the disease. In support of this view CT scanning of the pituitary region revealed an empty or partially empty sella in all the eight children with SCD who did not mount an appropriate GH response to clonidine stimulation. This might be due to hypoxic injury or infarction to the pituitary gland during one or more of the sickling episodes. In our child with severe SCD, according to severity index of SCD,[12] and empty sella with cisternal herniation Fig. 3, there was marked reduction in his GH response to high dose clonidine (7 μg/l) and to insulin-induced hypoglycemia (6.5 μg/l). This finding supports our hypothesis of hypoxic pituitary insults in these children.

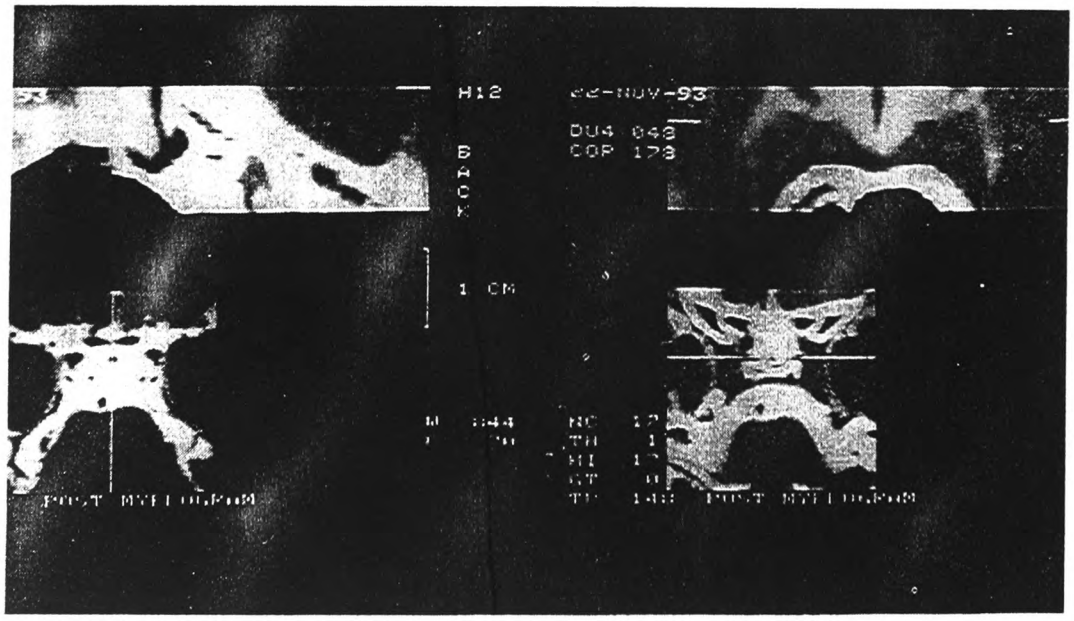

FIG. 3. CT after intrathecal contrast examination in a 12-year-old girl with SCD: sagittal reconstruction (left) and coronal reconstruction (right). Both show gross herniation of the suprasellar cisterna.

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Metabolism
Clinical and Experimental

VOL 46, NO 11 NOVEMBER 1997

Growth Hormone Secretion and Circulating Insulin-Like Growth Factor-I (IGF-I) and IGF Binding Protein-3 Concentrations in Children With Sickle Cell Disease

Ashraf T. Soliman, Nagwa El Banna, Issa AlSalmi, Vasantha De Silva, Alan Craig, and Maurice Asfour

Impaired growth involving both height and weight accompanying sickle cell disease (SCD) poses diagnostic and therapeutic problems. We undertook this study to test the hypothesis that this impaired growth is associated with abnormalities of the growth hormone (GH)/insulin-like growth factor-I (IGF-I)/IGF binding protein-3 (IGFBP-3)axis in 21 children with SCD and that SCD is associated with GH resistance. Nine of 21 children with SCD had a defective GH response to both clonidine and glucagon provocation (peak, < 10 μg/L); these children differed from the 12 others in having slower linear growth velocity (GV and GVSDS), lower circulating concentrations of IGF-I and IGFBP-3, and either partial or complete empty sellae in computed tomographic scans of the hypothalamic-pituitary area. In this group of patients with SCD, it appears that defective GH secretion and consequent low IGF-I production are the major etiological factors causing the slow growth. The two groups with SCD did not differ significantly in dietary intake, body mass index (BMI), midarm circumference, skinfold thickness, serum albumin concentration, or intestinal absorption of D-xylose. A single injection of GH produced a smaller increase in circulating IGF-I in children with SCD with or without defective GH secretion versus 10 age-matched children with idiopathic short stature (ISS) and 11 children with isolated GH deficiency (GHD), suggesting partial GH resistance in the SCD group. The presence of defective GH secretion, decreased IGF-I synthesis, and partial resistance to GH in short children with SCD suggests that treatment with IGF-I may be superior to GH therapy for improving growth.

GROWTH AND MATURATIONAL DELAY are striking features of sickle cell disease (SCD).[1] After 6 months of life, affected children have impaired growth involving both height and weight.[2-4] Many factors have been implicated in the etiology of growth impairment in children with SCD. These include chronic anemic hypoxia, increased energy expenditure due to high erythropoietic turnover and cardiac work,[5] nutritional deficiencies including zinc, folic acid, and vitamin A,[6-8] disturbed calcium metabolism,[9] repeated infections due to defective immune functions,[10-11] and dysfunction of the endocrine glands.[12]

The basal circulating concentrations of different hormones have been studied by different groups,[12-14] with no consensus for defining the endocrine abnormalities of the growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis and their possible contribution to growth impairment in these children.

Recently, we have reported a high incidence of defective clonidine-induced GH secretion and low circulating IGF-I concentrations in children with SCD and short stature.[15] IGF-I is a GH-dependent polypeptide that has a threefold function as a mediator of the growth-promoting action of GH, as a potent mitogenic factor, and as a metabolic regulator with insulin-like activity.[16-18] Alteration of IGF-I regulation may provide an attractive explanation for SCD-associated growth impairment.

The predominant IGF binding protein (IGFBP) in the blood is IGFBP-3, which forms a large 150-kd ternary complex. The serum level of this complex determines the total concentration of circulating IGF-I and regulates its growth-promoting potential.[19-25] Current opinion favors GH as the major regulator of IGFBP-3 and IGF-I levels in humans.[26] In vivo and in vitro experiments indicate relatively poor control of IGFBP-3 concentrations by IGF-I.[27-30] In addition, serum levels of IGF-I and IGFBP-3 are positively related to nutritional status.[27,31-33] No study to date has examined the different components of the GH/IGF-I/IGFBP system in children with SCD.

Treatment of SCD children with marked growth retardation using human GH may also be compromised by an associated GH resistance due to hepatic iron overload, malnutrition, or

From the Departments of Pediatrics, Endocrinology, and Chemical Pathology, Royal Hospital, Muscat, Oman; and SCL Bioscience Services, Cambridge, UK.

Submitted May 28, 1996; accepted April 28, 1997.

Address reprint requests to Ashraf T. Soliman, MD, Associate Professor of Pediatrics, Pediatric Endocrinologist, University of Alexandria, 3 Abdel Sattar Mansour St., Loran, Alexandria, Egypt.

0026-0495/97/4611-0001$03.00/0

repeated infections.[34,35] The IGF-I generation test provides valuable information on IGF-I sensitivity.[36]

We undertook the present study to test the hypothesis that (1) SCD is associated with abnormalities of the GH/IGF-I/IGFBP axis and (2) SCD is associated with GH resistance. The first issue was addressed by measuring the GH response to clonidine and glucagon and the circulating concentrations of IGF-I and IGFBP-3 in 21 children with SCD, and the second issue was tested using IGF-I generation tests. The results were compared with those for children with idiopathic short stature ([ISS] n = 10) and isolated GH deficiency ([GHD] n = 11).

SUBJECTS AND METHODS

Twenty-one prepubertal children with SCD were the subjects of this study. They were randomly selected, using random tables, from 58 children with SCD and short stature (height, 10th percentile for age and sex). The 58 children were all the patients with SCD and stature less than the 10th percentile selected from a cohort of 156 children with SCD regularly attending the hematology outpatient clinic of the Royal Hospital in Muscat, Oman. According to peak GH responses to two provocation tests (with clonidine and glucagon), they were divided into two subgroups. Nine of them (group 1) had a low peak GH response on at least two provocation tests (<7 μg/L), and the other 12 children (group 2) had a normal GH response to stimulation (>7 μg/L). All have been on folic acid supplementation and vaccinated against pneumococci. None of them had a history of intrauterine growth retardation or any other systemic or endocrine disease, dysmorphic trait, or central nervous system irradiation. Ten age-matched children with idiopathic short stature (ISS) (height standard deviation score [HtSDS] < −2 and normal GH response to provocation) and 11 children with isolated GHD (GH peak response < 5 μg/L on at least 2 provocation tests) served as controls. Informed consent was obtained from all the parents and, when appropriate, from the children before inclusion in the study.

All of the children were examined thoroughly, with special emphasis on the nutritional data. Auxanological measurements included weight, height, head circumference, midarm circumference, and scapular, triceps, and abdominal skinfold thickness. Harpenden calipers were used. The data recorded were the average of three sequential measurements determined by the same observer (A.T.S.). HtSDS was calculated according to the formula, HtSDS = (X1 − X2)/SD, where X2 and SD are the age-matched population mean height and SD, respectively, and X1 is the subject height.

Height growth velocity (GV) in centimeters per year was calculated for the previous 2 years. Normal population data were from Tanner et al.[37] Body mass index (BMI) was calculated according to the formula, BMI = weight (kg)/height (m)^2. Bone age was determined according to the Greulich and Pyle atlas.[38]

Children were admitted to the Royal Hospital in Muscat, Oman, for the period of investigation. The initial nutritional evaluation, both qualitative and quantitative, was performed by the dietitian using the recall method, and the patients received a high-protein (2 g/kg/d) diet for 7 days before hormonal evaluation. After an overnight fast (8 hours), venous blood samples were obtained between 8 and 9 AM for determination of complete blood cell count, serum albumin, bilirubin, alanine aminotransferase (ALT), bone-specific alkaline phosphatase (ALP), calcium, phosphorus, and bicarbonate concentrations. The serum was separated from the formed elements by centrifugation and kept frozen at −20°C until analyzed for IGF-I, IGFBP-3, cortisol, free thyroxine (fT_4), and thyrotropin (TSH) by radioimmunoassay. The subjects received a single injection of recombinant human GH 0.1 mg/kg subcutaneously. Serum IGF-I and IGFBP-3 levels were remeasured 24 hours after the injection.[36]

Human GH and IGF-I levels were measured by radioimmunometric assay using reagents purchased from Nichols Institute (San Juan Capistrano, CA). Intraassay coefficients of variation (CVs) were 5.6% and 6.8%, respectively, and interassay CVs were 7.8% and 8.3%, respectively, in the range of GH and IGF-I values detected. IGFBP-3 concentration was measured by radioimmunoassay at SCL Bioscience Services using reagents supplied by Mediagnost. The assay sensitivity was 0.06 μg/mL, with intraassay and interassay CVs of 5.2% and 8.6%, respectively.

Results are expressed as the mean ± SD and analyzed by ANOVA to compare analyte concentrations among groups. A paired Student *t* test was used to compare data in the same group before and after GH injection. Correlations between variables of interest were examined by linear regression analysis and, when appropriate, multiple regression analysis.

RESULTS

Children with SCD and ISS had normal circulating concentrations of albumin, calcium, phosphate, ALP, ALT, and bicarbonate. Serum levels of fT_4, TSH, and 8 AM cortisol did not differ among the two groups. Bilirubin (unconjugated) concentrations were higher and hemoglobin and hematocrit values were lower in the SCD group versus the ISS group. The peak D-xylose concentration after ingestion of 5 g D-xylose did not differ between the two groups.

Table 1 shows auxanological and hormonal data for children with SCD, GHD, and ISS. The HtSDS was significantly lower in children with ISS and GHD versus children with SCD. Linear

Table 1. Auxanological and Hormonal Data of the Three Groups

Group	Age (yr)	BMI (kg/m^2)	IGF-I-B (ng/mL)	IGF-I-A (ng/mL)	IGF-I-D (ng/mL)	IGFBP-B (mg/L)	IGFBP-A (mg/L)	IGFBP-D (mg/L)	Peak GH (μg/L)	HtSDS	GV (cm/yr)	GVSDS
SCD (n = 21)												
Mean	6.99	14.6	113	141*	27.6*†	1.86*	2.27	0.33*	7.36*	−1.34	4.9	−1
SD	3.27	1.8	78	74	15.6	0.8	0.87	0.258	3.85	0.6	1.7	1
ISS (n = 10)												
Mean	8.1	13.8	138	192	53.5†	2.39	2.42	0.133	19.6	−2.3*	4.6	−1.1
SD	1.8	0.6	43	39	17.6	0.37	0.36	0.071	2.7	0.3	0.4	0.5
GHD (n = 11)												
Mean	7.3	15.1	66.1†	146.1	82.3	ND	ND	ND	4.6†	−3.2†	3.7†	−2.3
SD	1.8	1.2	25	50.7	42.4				1.9	0.9	1.2	1.2†

NOTE. Plasma IGF-I and IGFBP-3 were measured immediately before (B) and 24 hours after (A) GH administration (0.1 mg/kg). The suffix "-D" indicates the change in IGF-I and IGFBP-3 from basal to 24 hours.

*$P < .05$, SCD *v* ISS.

†$P < .05$, SCD *v* GHD.

GV, GVSDS, and BMI did not differ significantly among the two groups with ISS and SCD. However, GV and GVSDS were significantly decreased in children with GHD. The peak GH response to provocation was significantly lower in children with SCD versus ISS ($P < .001$). Basal circulating IGF-I concentrations were higher in children with ISS versus the other two groups. Basal circulating IGFBP-3 concentrations were significantly lower in the SCD group ($P < .05$) versus the ISS group. The IGF-I response to GH administration ([IGF-I-D], equal to the 24-hour IGF-I value minus the basal value) was significantly lower in children with SCD (27.6 ± 15.6 ng/mL) compared with children with ISS (53.5 ± 17.6 ng/mL). IGF-I increased significantly after human GH injection in the three study groups. However, the IGF-I response (IGF-I-D) was significantly higher in children with GHD and those with ISS versus children with SCD.

Table 2 presents a comparison between two groups of children with SCD: group 1 with defective GH secretion (n = 9; peak GH response to provocation, 4.2 ± 2.3 μg/L) and group 2 (n = 12) with a normal peak GH response to provocation (9.9 ± 2.4 μg/L). Age and BMI did not differ among the two groups. HtSDS and GVSDS were significantly lower in group 1. Basal circulating concentrations of IGF-I and IGFBP-3 were significantly lower in group 1 (SCD with defective GH secretion). IGF-I responses to GH administration did not differ significantly among the two groups and were significantly lower than those for children with isolated GHD.

Correlations between auxanological and hormonal data in 21 children with SCD are presented in Table 3. Basal and GH-stimulated concentrations of IGF-I and IGFBP-3 were correlated significantly with height GV and GVSDS ($P < .01$) and GH peak values. The dependence of serum IGFBP-3 on GH was evident from a significant correlation with peak GH secretion (n = 21, $r = .665$, $P < .01$) and the significant increase in IGFBP-3 after one injection of GH. IGFBP-3 concentrations correlated significantly with IGF-I levels before and after GH administration ($r = .600$, $P < .01$). Serum ferritin was inversely correlated with GV, GVSDS, and IGF-I ($r = -.35$, $-.46$, and $-.25$, respectively, $P < .05$).

Table 2. Auxanological and Hormonal Data of Children With SCD Versus Those With GHD

	Age (yr)	BMI (kg/m²)	IGF-I-B (ng/mL)	IGF-I-A (ng/mL)	IGF-I-D (ng/mL)	IGFBP-B (mg/L)	IGFBP-A (mg/L)	IGFBP-D (mg/L)	Peak GH (μg/L)	HtSDS (−)	GV (cm/yr)	GVSDS (−)
SCD + GHD (n = 9)	6.6 ± 2.7	14.8 ± 2.2	46.1 ± 30.7*	78 ± 36*†	29.8 ± 17.1†	1.13 ± 0.37*	1.42 ± 0.32*	0.30 ± 0.26	4.2 ± 2.3*	1.68 ± 0.79	4.1 ± 1.5*	1.55 ± 0.5*
SCD (n = 12)	7.3 ± 3.4	14.4 ± 1.3	136 ± 59*	189 ± 55	25.9 ± 14†	2.4 ± 0.5*	2.9 ± 0.5*	0.35 ± 0.25	9.9 ± 2.4†	1.1 ± 0.9	5.4 ± 1.5	1.1 ± 0.68
GHD (n = 11)	7.3 ± 1.8	15.1 ± 1.2	66.1 ± 25	146.1 ± 50	82.3 ± 42.4*	ND	ND	ND	4.6 ± 1.9	3.2 ± 0.9†	3.7 ± 1.2†	2.3 ± 1.2†

NOTE. Data are the mean ± SD. The suffix "-D" indicates the change in IGF-I and IGFBP-3 from basal to 24 hours.
*$P < .05$, SCD *v* SCD + GHD.
†$P < .05$, SCD groups *v* GHD.

DISCUSSION

There is no completely reliable test for diagnosing or excluding GHD in all short children. When used alone, none of the tests have superior diagnostic specificity or sensitivity. Measurement of GH-response peptides such as IGF-I and IGFBP-3 can add insights even when the results do not agree with GH responses to provocative stimuli. Interpretation of the tests together improves the reliability of diagnostic assessment.[39]

Nine of 21 randomly selected children with SCD (43%) had defective GH secretion in response to provocation with both clonidine and glucagon. In these children, IGF-I concentrations were comparable to those in children with isolated GHD, but were significantly lower than those in children with ISS and normal GH release. Their circulating IGFBP-3 concentrations, which reflect the integrated GH secretion over a period of days,[26,39-41] were significantly lower versus the group with normal GH secretion, but were not different from those reported by Smith et al[39] for children with GHD. The linear growth of

Table 3. Correlations Between Auxanological and Hormonal Data (r values)

	Peak GH (μg/L)	GV (cm/yr)	GVSDS	BMI (kg/m²)
IGF-I-B	.483†	.4†	.529†	−.3*
IGF-I-A	.436†	.476†	.538†	−.24
IGF-I-D	−.505†	−.002	−.08	−.31*
IGFBP-3-B	.559†	.2	.25*	−.27*
IGFBP-3-A	.716†	.25	.35*	−.15
Ferritin	−.175	−.36*	−.46†	−.01
Peak GH	1	.337*	.339*	.113

Abbreviations: B, before GH; A, after GH.
*$P < .05$.
†$P < .01$.

patients with SCD with defective GH secretion was significantly slower than that of patients with normal GH secretion. This defective GH secretion may be secondary to a hypoxic vascular insult to the hypothalamic-pituitary area during one or more of the sickling episodes and/or to pituitary atrophy as a result of iron overload. In support of this view, computed tomographic scanning of the pituitary region revealed empty sellae (partial or complete) in all nine children with SCD and GHD. Moreover, the SCD severity score, as described by El Hazmi et al,[12] was significantly higher in the SCD group with defective GH secretion versus those with normal GH secretion. Collectively, these findings suggest that acquired GHD may be a major factor in the etiology of retarded growth in some patients with SCD, especially those with severe sickling attacks.

It is unclear which of the alterations of the GH/IGF-I/IGFBP axis found in children with SCD are of greater clinical significance. Although the influence of SCD on adult height is controversial, most data indicate a negative effect of SCD on linear growth.[1-4,6] In our study, the mean HtSDS for all patients with SCD (n = 21, −1.34 ± 0.6) was in the lower range of normal. SCD patients with defective GH secretion had a significantly lower HtSDS and GV versus those with normal GH release. Moreover, we found a good correlation between GVSDS and IGF-I (before and after GH injection) and a strong correlation between the peak GH response to provocation and IGF-I and IGFBP-3. These findings suggest that circulating IGF-I and IGFBP-3, as in normal children, are the major determinants of linear growth in children with SCD, and both are regulated by the GH status of the child. On the other hand, the degree of GH resistance as measured by the change in IGF-I concentration after GH injection (IGF-I-D) was not correlated with linear growth parameters (HtSDS, GV, and GVSDS, $r = -.26, -.002$ and .083, respectively) in these children. This may denote that GHD plays a more significant role than GH resistance in the etiology of impaired linear growth in these children.

In children with SCD, growth impairment includes both height and weight.[2-4] Treatment with human GH has been shown to improve nitrogen balance and linear growth in a variety of growth disorders.[42] The detection of defective GH secretion in about 40% of slowly growing children with SCD suggests a beneficial growth-promoting effect of GH therapy in these patients. The IGF-I generation test has been used to assess IGF-I responsiveness to GH in children with short stature.[36] In this study, a single subcutaneous injection of GH has been used to investigate the physiological changes of IGF-I/IGFBP-3 in children with SCD to test the hypothesis that SCD is associated with GH resistance. Subjects with SCD with or without defective GH secretion had significantly lower IGF-I responses to a single injection of GH versus children with ISS and those with isolated GHD, suggesting partial GH resistance. This might attenuate the anabolic effects of GH therapy in these children.[34,35] The IGF-I response (IGF-I-D) correlated negatively with the BMI ($r = -.31$, $P < .01$), suggesting that in children with SCD, increasing wasting may be associated with progressive GH resistance. The significant negative correlation between the IGF-I response to GH injection and the peak GH response to provocation ($r = -.505$, $P < .01$) suggests that IGF-I production is better in children with defective GH secretion. However, the IGF-I response was nonsignificantly higher in children with SCD and GHD versus those with normal GH secretion.

Malnutrition can be excluded as an important cause of abnormalities of the GH/IGF-I axis in children with SCD because of the following: (1) BMI, subcutaneous fat thickness, midarm circumference, and serum albumin concentration were not different among the study groups (SCD, SCD + GHD, and ISS); (2) analysis of the nutritional history showed adequate qualitative and quantitative intake of nutrients as compared with 20 normal age-matched children; (3) the D-xylose test was normal in all children with SCD and those with ISS; and (4) in malnutrition, basal GH concentrations are usually high, unlike the case in our children with SCD (mean ± SD, 1.8 ± 0.25 μg/L; range, 0.5 to 3.5).[43]

The normal serum levels of creatinine, bicarbonate, calcium, phosphorus, and ALP exclude any significant contribution of renal dysfunction and/or disturbed calcium homeostasis in the etiology of growth impairment or endocrine abnormalities in these children. Normal serum concentrations of albumin, ALP, and ALT with a normal albumin to globulin ratio and prothrombin time in children with SCD make it unlikely that impaired hepatocellular synthetic function is a cause of the low IGF-I production in these patients.

The serum ferritin concentration, as an indicator of body iron stores, was negatively correlated with the height GV ($r = -.35$, $P < .01$), GVSDS ($r = -.464$, $P < .01$), and circulating concentration of IGF-I ($r = -.255$, $P < .05$). This suggests that hepatic/parenchymal iron overload may impair IGF-I synthesis and subsequently slow linear growth in children with SCD.

In summary, children with SCD have significantly lower IGF-I production in response to GH injection versus children with ISS and GHD, suggesting partial GH resistance. Some children with SCD and delayed growth may have GHD. Parenchymal iron overload, a potentially treatable factor, may contribute to the etiology of impaired hepatic IGF-I synthesis and/or defective GH secretion by the pituitary gland. Because of the presence of partial GH resistance, treatment with recombinant IGF-I may be more successful than treatment with GH.

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Original Article Indian J Pediatr 1995; 62 : 75-1

Empty Sella in Short Children With and Without Hypothalamic-Pituitary Abnormalities

Ashraf T. Soliman, Assim Darwish* and Maurice G. Asfour**

Department of Pediatrics, Radiology and Endocrinology** Royal Hospital, Muscat Sultanate of Oman*

Abstract. A study was conducted on growth hormone (GH) response to oral clonidine (0.15 mg/m²), GH and cortisol responses to i.m. glucagon (0.1 mg/kg), and glucose response to an oral load of glucose (1.75 g/kg). Measurements were made on the circulating concentrations of free thyroxine (FT4), thyroid stimulating hormone (TSH) and different growth parameters and CT sellar images in 25 GH deficient children (Peak GH response to clonidine and glucagon < 7 ug/ml), 15 growth retarded children (Ht < 5th percentile for age and gender) with sickle cell disease (SCD) and GH deficiency, 30 randomly selected children with normal variant short stature (NVSS) (HtSDS 2SD below the mean for age and gender with normal GH response to stimulation (> 10 ug/ml) and 20 age-matched normal children were evaluated. Out of the 25 children with GH deficiency, five had multiple pituitary hormonal deficiency (GH, TSH and/or ACTH. deficiencies), and 20 had isolated GH deficiency. Empty sella, either complete or partial, was detected in 9 out the 20 children with isolated GH deficiency (45%), 4 out of the 5 children with multiple pituitary deficiency (80%), all the children with SCD and GH deficiency (100%), 3 out of the 30 children with NVSS (10%) and in none of the normal children. The insulin-like growth factor-I (IGF-I) concentrations were significantly lower in the two groups of children with GH deficiency compared to those with NVSS. The height standard deviation scores (HTSDS) were significantly lower and the annual growth velocity was slower in children with idiopathic GH deficiency and empty sella compared to those with NVSS and those with empty sella associated with SCD. The bone age delay (yr) did not differ among the 3 groups of children with short stature. All children with isolated GH deficiency associated with empty sella had normal body mass indices (BMI), while all the children with SCD and empty sella had BMI below the 5th percentile for the corresponding age and gender. None of the children had glucose intolerance. In conclusion, children with growth retardation and abnormal hypothalamic pituitary functions have high incidence of empty sella. However, empty sella is detected in considerable number (10%) of short children with normal hypothalamic pituitary function. (Indian J Pediatr 1995; 62 : 75-1)

Key words :

For sella most frequently radiographic studies are undertaken on children as a part of the investigation of growth disorders and in them as many as one fourth is found to have an empty sella.[1] The term empty sella syndrome (ESS) refers to the herniation of the supraseller subarachnoid spaces in the sella turcica with various degrees of flattening of the pituitary. Empty sella is classified as primary (idiopathic), or secondary to pituitary tumors, surgical or radiation therapy and, probably, to pituitary hypoplasia. In adults, empty sella is

Reprint requests: Dr. Ashraf T. Soliman, Chief, Pediatric Endocrinology, Royal Hospital, Seeb Airport 1331, Muscat, Sultanate of Oman.

a relatively common finding and is often asymptomatic. In contrast, cases reported in children are more often associated with endocrine abnormalities in 25-45% of patients. Endocrine abnormalities reported include growth hormone (GH) deficiency, diabetes insipidus, sexual precocity, hyperprolactinemia and panhypopituitrism.[2,3] In most of the reported series the patients have enlarged sella turcica with more than 50% of the sella replaced with CSF. The finding of normal size sella (less than 50% of the sella being replaced with SCF) has been considered a normal variant by many radiologist. However, recently Michaels *et al*[4] reported significant endocrine abnormalities in patients with normal size empty sella.

Here are reported the growth parameters, static and dynamic pituitary function testing and result of CT evaluation of the hypothalamic-pituitary area in 25 children with GH deficiency, 15 children with sickle cell disease (SCD) and GH deficiency, 30 age-matched children with normal variant short stature (NVSS) and 20 normal age-matched normal children.

Material and Methods

Ninety prepubetral (Tanner stage 1) children with age range between 4 and 10 years were the subjects of this study. They included : (A) 25 children with GH deficiency (5 with multiple pituitary deficinecy including GH, TSH and ACTH, and 20 with isolated GH deficiency), they were all the diagnosed cases in the endocrine clinic of the Royal Hospital, Muscat, Oman between April 1991 and August 1993, (B) 15 children with SCD and GH deficiency, they were diagnosed out of 34 children with SCD and growth retardation presented to the same clinic during the same period, (C) 30 children with NVSS (HtSDS > 2SD below the mean for age and gender) who were randomly selected from all the children with NVSS who attended the endocrinology clinic and investigated between January 1992 and January 1993 (30 out of 95), and (D) 20 age-matched children with normal stature (HtSDS = +/-1), who were selected from the radiology department while taking CT scans for other reasons (15 wit head trauma to role out intracranial lesions and 5 to role out intraocular pathology). All of them had no pathology in their CT. All the children with SCD were on regular blood transfusion to keep their hemoglobin (Hb) concentrations above 9g/dl and folic acid supplements. None of the children in this study had history of intrauterine growth retardation, malnutrition, any other systemic disease, dysmorphic trait, encephalsits, intracranial lesions or central nervous system irradiation. Informed consent for the testing procedure and CT Scanning was obtained from the parents of all children. CT scans of the brain with 1 mm cuts in the hypothalamic pituitary area were evaluated by the team Neuroradiologist (AD). Empty sella was defined as sella that, regardless of its size, is completely or partly filled with CSF.[5,6] The anthropometric measurements including weight (Wt) and height (Ht) were recorded using the Harpenden's anthropometric measuring instruments. The data recorded were the average of three sequential measurements determined by the same observed (AST). The HtSDS was calculated according to the formula HtSDS= (X1-X2)/SD where X2 and SD are age-matched population mean height and SD respectively and X1 is the subject height. The height growth velocity

(GV) cm/yr and GV standard deviation scores (GVSDS) were calculated for the last year. Normal population data were according to Tanner *et al.*[7] The body mass indices (BMI) were calculated according to the formula BMI = Wt (kg)/Ht (m)^2. The bone age was determined according to Greulich and Pyle atlas.[8]

On the day of admission, venous blood samples were obtained for determination of complete blood count (CBC) and serum albumin, bilirubin, alanine transferase (ALT), alkaline phosphatase (ALP), calcium, phosphorus and bicarbonate concentrations. Following an overnight fast (8-h) venous blood samples were withdrawn through a polyethylene catheter inserted in a forearm vein between 8 and 9 a.m. The serum was separated from the formed elements by centrifugation and kept frozen at -20°C until analyzed for GH, free T_4 (FT_4), TSH, and IGF-I. After obtaining the basal samples, an oral dose of clonidine (0.15 mg/m^2) was given and venous blood collected every 30 minutes for 2 hours for measurement of serum GH concentrations. On the next morning and after an 8-h overnight fast 0.1 mg of lucagon (max 1 mg) was injected and i.m. and blood samples obtained before and every 30 min after the injection for 180 min for estimation of GH and cortisol concentrations.[9] On the third day and after an overnight fast oral glucose 1.75 gm/kg (20% solution) was given to the child and blood collected before and 120 minutes after the oral glucose load for determination of plasma glucose concentration by glucose oxidase method. Human GH and IGF-I concentrations were measured by radioimmunometric assay, employing reagents purchased from Nichols Institute (San Juan Capistrano, CA). Intra-assay coefficient of variations showed on average of 6% in the range of GH values detected and 5.7% in the range of IGE-I concentrations measured. All samples from the patients were assayed simultaneously. FT4, TSH and cortisol concentrations were measured using Amerlex-RIA kits, Kodak Clinical Diagnostics, Amersham, UK. Statistical analyses were done using ANOVA test to compare mean analyte concentrations among the study groups when the data were normally distributed and Wilcoxone test when they were not. Statistical significance was accepted at $p < 0.05$.

Results

Table 1 presents anthropometeric and bone age data of the patients and controls. The HtSDS was significantly lower in the 2 groups of children with GH deficiency compared to those with NVSS and normal children. Growth velocity was significantly slower and GVSDS was lower in children with GH deficiency vs those with SCD+GH deficiency. The bone age was significantly delayed in the 3 groups of children with short stature compared to the controls. However, the degree of delay did not differ among the 3 groups.

The biochemical and hematological data of the 4 study groups are presented in Table 2. Creatinine clearance, and serum concentrations of albumin, ALT, ALP, bicarbonate, calcium, phosphate and albumin did not differ among the 4 study groups.

Table 3 shows the hormonal profile and glucose data of the 4 study groups. Out of the 25 children with GH deficiency, 20 (80%) had isolated GH deficiency, and 5 (20%) had multiple pituitary deficiency (GH, TSH and/or ACTH). All the growth

TABLE 1. Anthropometric and Bone Age Data of Patients and Controlds

Groups	Age (yr) (yr)	BMI kg/m²	HtSDS (-)	GV cm/yr	GVSDS (-)	B-age delay (yr)
GHD (n = 25)	7.7 +/-2.3	15.2 +/-1.9	3.6 +/-.4*	2.8 +/-1*	3.2 +/-1*	2.4 +/*-1.4*
NVSS (n = 30)	8.1 +/-1.5	13.8 +/-0.5	2.6 + /-.3*	4.6 +/-.04*	1.1 +/-0.5	2.25 +/-1*
SCD + GHD (n = 15)	8.6 +/-2.9	11.1+/-0.7*	3.1 +/-.5*	4.2 +/-0.9*	1.4 +/-0.5*	2.7 +/-1.2*
Controls (n = 20)	7.6 +/-0.7	15.7 +/-0.5	0.4 +/-0:1	6.6 +/-0.3	0.2 +/-.04	0.8 +/-0.2

GHD = GH deficiency, * $p < 0.05$ groups vs controls

TABLE 2. Biochemical and Hematological Data of the Short Children and Controls

Groups	Alb g/L	ALT IU/L	Bili umol/L	Ca mmol/L	Phos mmol/L	ALP IU/L	Cr Clearance	Bicab mmol/L	Hb g/L	Hct
GHD (n = 25)	42.7 +/-5	20.3 +/-6.3	9.2 +/-2.9	2.3 +/-0.1	1.4 +/-0.4	142 +/-34	115.5 +/-12	19.3 +/-3.7	11.2 +/-1.7	0.35 +/-0.06
SCD + GHD (n = 15)	43.8 +/-4.1	25 +/-7.8	23 +/-12	2.35 +/-0.1	1.6 +/-0.06	180 +/-54	100 +/-12	18.6 +/-1.6	8.9 +/-1.0	0.263 +/-0.03
NVSS (n = 30)	37 +/-4	21 +/-13	15+/-5	2.2 +/-0.1	1.4 +/-0.9	161 +/-25	120+/-10	22.2 +/-1.8	12.8 +/-1.1	0.37 +/-0.04
Controls (n = 20)	42 +/-1.5	16 +/-6	10 +/-3	2.3+/-0.08	1.3 +/-0.2	152 +/-35	111 +/-15	22 +/-1.2	132 +/-1.7	0.38 +/-0.04

GHD = Growth hormone deficiency; Alb = albumin; Cr clearance (ml/min/1.73 m²)

TABLE 3. Hormonal and Glucose Data in Patients and Controls

Goups		FT4 pmol/L	TSH ulU/ml	GH (B) ug/L	GH (P) ug/L	IGF-I IU/L	Cortisol (B) nmol/L	Cortisol (P) nmol/L	Glucose-O mmol/L	Glucose-120 mmol/L
GHD	Mean	15.7	1.2	2.2	5.6*	10.3*	623	1014	3	5.1
n = 25	SD	2.5	0.6	1.7	3	2.5	335	391	0.8	0.5
NVSS	Mean	17.4	1.6	1.6	19.6	22.1	466	1062	3.5	5.5
n = 30	SD	0.6	0.15	0.2	2	4.8	62	208	0.8	0.6
SDD + GHD	Mean	16.1	1.4	1.1	6*	9.2*	529	862	3.1	5.7
n = 15	SD	1.5	0.4	0.5	2.6	2	120	270	0.6	0.4
Controls	Mean	14.6	1.6	1.2	16.4	32.1	496	965	4.2	5.3
n = 20	SD	1.5	0.05	0.2	0.9	5.8	35	110	0.4	0.5

(b) = basal, (P) = peak after stimulation; * $p < 0.05$ groups vs controls

retarded children in the sickle cell group, who had defective GH response to stimulation (GH Peak < 10 ug/L), had empty sella (100%), and their FT4, TSH, 8AM cortisol concentrations and cortisol response to glucagon were normal. The IGF-I concentrations were significantly lower in the 2 groups of children with GH deficiency compared to those with NVSS and normal children. Empty sella was noted in 9 out of the 20 children with isolated Gh deficiency (45%), 4 out of the 5 children (80%) with multiple hypothalamic-pituitary hormonal deficiencies (none with diabetes insipidus), all the children with SCD associated with GH deficiency, 3 out of 30 children with NVSS (10%) and in none of the normal children. Children with isolated GH deficiency and ESS had normal BMI for age and gender (15.4 +/- 1.7), whereas children with SCD and ESS had BMI (11.1 +/-0.7) below the 5th percentile for corresponding age and gender. None of the children had glucose intolerance or high blood pressure.

DISCUSSION

In this study the incidence of empty sella was 45% in children with isolated GH deficiency, 80% in those with multiple pituitary deficiency, 100% in children with SCD and GH deficiency and 10% in the short children with normal hypothalamic-pituitary function. None of the children with normal stature had empty sella. These findings suggest that empty sella has a considerably high incidence in children with growth and hypothalamic pituitary disorders, particularly those with multiple pituitary hormone deficiency. This implicates the importance of CT scanning of the hypothalamic-pituitary area in children with severe short stature as an important

tool in the identification of children with pituitary hormonal deficiency . Our findings are in agreement with the study of surtees *et al*[10] who reported empty sella in 90% of patients with multiple pituitary deficinecy and in 37% of patients with isolated GH deficiency. However, Marwaha et al[11] reported empty sella in 16 out of 22 children affected by isolated GH deficiency born by normal delivery (72.7%). Such difference may be partly due to their use of MRI which is more sensitive in the diagnosis of empty sella. In addition, some of their patients (23%) had brain anomalies in the scan, a characteristic which was not found in the patients of present study.

The association of empty sella and diabetes insipidus has been reported previously in CT scans.[12,13] However, none of our patients with primary empty sella nor those with empty sella and SCD had diabetes insipidus. This finding is in agreement with those of Cacciaricet *et al*[14] who reported absence of diabetes insipidus in their patients (n = 23) with primary empty sella without other anatomical abnormalities. It seems that the presence of intrasellar CSF does not have an adverse effect on posterior lobe function.

The presence of empty sella in all the sickle cell children who did not mount normal GH response to pharmacological stimuli suggests that ischemic injury to the pituitary during the sickling episodes might be the cause of both GH deficiency and empty sella in these children. The hypothalamic-pituitary adrenal and thyroid functions were normal in these children.

None of the children with empty sella had symptoms or signs of increased intracranial pressure, obesity or glucose intolerance reported in adults with empty sella.[15] In the present study the significantly low BMI in children with SCD +empty sella and normal BMI in children with isolated GH deficiency and empty sella exclude an important effect, if any, of empty sella on the control of body weight in children.

In summary, empty sella is frequently seen in children with idiopathic GH deficinecy and children with GH deficiency secondary to SCD but it is not rare in very short prepubertal children with normal hypothalamic-pituitary function.

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Insulin and Glucagon Responses to Provocation with Glucose and Arginine in Prepubertal Children with Thalassemia Major Before and After Long-term Blood Transfusion

by Ashraf T. Soliman,* MD, Nagwa El Banna,** MD, Issa AlSalmi,** MB Ch B, *and* Maurice Asfour,** MD
**Department of Pediatrics, University of Alexandria, Alexandria, Egypt*
***Department of Endocrinology, Royal Hospital, Muscat, Oman*

Summary

Hypertransfusion therapy has dramatically increased the duration and quality of life in patients with B-thalassemia major; however, it leads to chronic iron overload, and is frequently complicated by the development of diabetes mellitus or impaired glucose tolerance. To determine the early effect of iron overload on the endocrine pancreatic function, we studied glucose, insulin, and glucagon responses to oral load of glucose and to arginine provocation in 15 children with B-thalassemia major, before and after (3.1 ± 0.6 years) high-transfusion and iron chelation and compared them with 15 age matched normal controls. In addition, we evaluated growth hormone (GH) responses to oral clonidine and measured the circulating insulin-like growth factor-I concentration in thalassemic children on long-term transfusion and controls. After long-term high-transfusion, thalassemic children had significantly decreased serum insulin concentrations and low insulin/glucose ratios at 60 and 120 min after an oral glucose load (1.75 g/kg) in comparison with values before therapy and those for controls. None of the thalassemic children had glucose intolerance after this period of frequent blood transfusion; however, their serum glucose levels at 60 and 120 min after the oral glucose load were significantly higher compared to control children. Thirty minutes after starting arginine infusion, serum insulin concentration was significantly lower in thalassemic children compared to before therapy. Basal and arginine-stimulated glucagon secretions were significantly elevated in thalassemic children on long-term blood transfusion with significantly low serum insulin/glucagon ratios. In addition, the high basal serum glucagon concentrations were not suppressed after the oral glucose load. Despite hyperglucagonaemia in all thalassemic children, their blood glucose dropped appropriately below 50 per cent of the fasting glucose level after an intravenous insulin dose (0.1 U/kg) ruling out any significant insulin-resistance. GH responses to clonidine provocation were subnormal in thalassemic children after long-term blood transfusion compared to controls. In summary, thalassemic children on long-term blood transfusion and iron chelation have progressive and early loss of B-cell mass, manifested by decreased insulin release in response to secretagogues, before the development of significant insulin resistance or impairment of glucose tolerance.

Introduction

In thalassemia major, frequent blood transfusions are required and this leads to massive iron overload.[1] The duration of the disease and the number of transfusions are highly correlated with the degree of glucose intolerance.[2–6] The reported prevalence of diabetes in treated thalassemia major is about 16 per cent, while the incidence of impaired glucose tolerance approximates 60 per cent.[7] Possible pathogenic conditions are pancreatic cell destruction with subsequent insulin deficiency,[3,4,6] liver derangement with consequent insulin resistance,[8–10] genetic factors,[2,6] and possible immunological disturbance.[11] In addition, 28 per cent of these patients develop diabetes mellitus shortly after an acute viral hepatitis infection, suggesting an important role of hepatitis virus in precipitating a diabetic state in these patients.[12]

Data about the circulating concentrations of insulin in non-diabetic children with thalassemia are controversial. Toccafondi R *et al.*[13] reported lower insulin response to oral glucose in their thalassemic children with normal oral glucose tolerance (GTT) and Lassman *et al.*[3,14] found a delayed insulin

Correspondence: Dr Ashraf T. Soliman, Pediatric Endocrinology, Royal Hospital, P.O. Box 2331, Seeb, Muscat, Code 111, Oman.

response to oral glucose loading. In controversy, Flynn *et al.*[5] and Merkel[15] described high insulin level in patients with normal GTT and suggested the possibility of insulin-resistance. Kuo *et al.*[16] found that in only 50 per cent of older thalassemic patients did the plasma glucose fall to less than half of the fasting levels after intravenous (iv) insulin, whereas Toccafondi *et al.*[17] found that plasma glucose fell normally after (iv) insulin in thalassemic children under 10 years.

Diabetic hyperglycaemia is generally believed to be a bihormonal disorder where absolute or relative lack of insulin and excess of glucagon cause decreased peripheral glucose uptake and increased hepatic glucose production.[18–22] In agreement with this notion, increased hepatic glucose production and plasma glucagon concentrations have been demonstrated to correlate with the hyperglycemic level.[19–25]

Lassman *et al.*[3] found diminished glucagon response to intravenous alanine infusion in thalassemic patients without family history of diabetes and suggested that this is due to iron loading on the alpha cells. In controversy, Passariello[26] and Nelson *et al.*[27] demonstrated elevated glucagon level in thalassemic and hemochromatic patients.

In our prospective study we have evaluated insulin and glucagon secretion in response to oral glucose and arginine infusion in young thalassemic children before and after long-term (3.1 ± 0.6 years) high-transfusion and iron chelation. In addition, growth hormone (GH) and cortisol responses to insulin-induced hypoglycemia were evaluated, and serum concentrations of insulin-like growth factor-I (IGF-I), free thyroxine (FT4), and TSH measured in thalassemic children after long-term high-transfusion and results compared to an age-matched normal group of children.

Subjects and Methods

Twenty young children with thalassemia major, diagnosed between January 1988 and January 1990, were selected from the Hematology clinic of Alexandria University Children's Hospital for the study. Their ages ranged between 1.75 and 3.5 years (mean 2.8 ± 0.6 years). None had family history of diabetes mellitus, intra-uterine growth retardation, or any other systemic or endocrine disease. Informed consent was obtained from all the parents of the children before including in the study. The Ethical Committee of Alexandria University approved the study protocol. Before receiving the first blood transfusion, children were admitted and offered high carbohydrate diet for 3 days. On the 4th day and after an 8-hour overnight fast a standard oral glucose tolerance test was performed and blood collected at 0, 60, and 120 min after the oral glucose load (1.75 g/kg) for estimation of glucose concentration by glucose oxidase method, and glucagon and insulin concentrations by radio-immunoassay using kits purchased from Amersham. On the 5th day and after an overnight fast an intravenous (iv) infusion of 10 per cent arginine HCl infusion (0.5 g/kg) was started and continued for 30 min and serum samples obtained before and at 30 and 60 min after the start of the infusion for estimation of glucose, insulin, and glucagon concentrations.

Children were started on frequent transfusion therapy (15 ml of packed erythrocytes/kg of body weight given every 4 weeks to maintain the haemoglobin level at 9–10 g/dl) and deferoxamine (50 mg/kg/dose for up to 6 days per week) given by intramuscular injection. During each clinic visit the children were examined, and nutritional and growth data, as well as their anthropometric measurements including weight and height recorded. The Height standard deviation score (HtSDS) was calculated according to the formula $\text{HtSDS} = (\bar{X}1 - \bar{X}2)/\text{SD}$, where $\bar{X}2$ and SD are age-matched populations mean height and SD, respectively, and $\bar{X}1$ is the subject's height. The height growth velocity (GV) cm/year and body mass index (BMI) were calculated for each year and recorded. Normal population data were according to Tanner *et al.*[28] Laboratory investigations included complete blood count (CBC) and estimation of serum concentration of albumin, bilirubin, alanine transferase, urea, and creatinine at 6-month intervals.

After a period of 2.5–4 years ($\text{mean} = 3.1 \pm 0.6$ years) of high-transfusion and chelation 15 out of the 20 patients were admitted and the previous hormonal investigations redone. Three children were excluded from the study because of poor compliance and the other two because parents refused to give consent for retesting. In addition, after an overnight fast, a basal serum sample was collected for measurement of GH, cortisol, IGF-I, FT4, and TSH concentrations. GH response to oral clonidine (0.15 mg/m^2) was evaluated in all the thalassemic children and serum collected at 0, 60, and 90 min after clonidine intake for estimation of GH. Fifteen normal age-matched children served as controls for the hormonal and biochemical studies after taking parents' consents. GH and IGF-I were measured by radio-immunometric assay, employing reagents purchased from Nichols Institute (San Juan Capistrano, CA). Intra-assay coefficient of variation averaged 6.7 per cent in the range of GH values detected and 8.1 per cent in the range of IGF-I concentrations measured. FT4, TSH, and cortisol were measured using Amerlex-RIA kits, Kodak Clinical Diagnostics, Amersham, UK. Skeletal age was determined according to the Atlas of Greulich and Pyle.[29]

Statistical analyses were done using the ANOVA test to compare analyte concentrations before versus after long-term blood transfusion when the data were normally distributed and Wilcoxon test when they were not. Statistical significance was accepted at $P < 0.05$.

Results

Glucose, glucagon, and insulin data are presented in Tables 1 and 2. During fasting, serum insulin, and glucose concentrations did not differ among the study groups. Serum glucagon was significantly higher in thalassemic children after compared to before frequent blood transfusion commenced. After oral glucose load insulin secretion was significantly reduced in thalassemic children after *v.* before long-term high-transfusion. Their serum insulin/glucose (I/G) ratios were significantly lower [(0.27 ± 0.12) at 1 h and (0.28 ± 0.13) at 2 hours] *v.* before therapy [(0.62 ± 0.26) at 1 hour and (0.55 ± 22) at 2 hours]. In thalassemic children on long-term blood transfusion serum glucagon was not suppressed after an oral glucose load. Serum glucagon levels were significantly higher at 60 and 120 min after the oral glucose load compared to values before therapy and to those in the control children. None of the thalassemic children had impaired glucose tolerance after this period of frequent blood transfusion and chelation; however, their serum glucose levels were significantly higher at 60 and 120 min after the oral glucose load, compared to those for the control group. Thirty minutes after starting arginine infusion serum insulin concentration was significantly lower in thalassemic children after long-term high-transfusion *v.* before therapy. Basal and arginine-stimulated glucagon concentrations were significantly higher in thalassemic children on frequent blood transfusion *v.* before therapy. Consequently, serum insulin/glucagon ratios at 0, 30, and 60 min after starting arginine infusion were significantly decreased in thalassemic children after (0.10 ± 0.06, 0.08 ± 0.05 and 0.11 ± 0.07) *v.* before therapy (0.15 ± 0.07, 0.17 ± 0.08, and 0.15 ± 0.07).

Table 3 presents growth parameters and hormonal data of thalassemic children after a mean transfusion period of 3.1 ± 0.6 years. The HtSDS of thalassemic children decreased significantly from −0.75 ± 0.3 before starting blood transfusion to −1.65 ± 0.5 at the time of retesting. HtSDS, GV, and BMI were significantly lower in thalassemic children compared to controls. Serum ferritin, bilirubin and ALT concentrations were significantly higher in thalassemics *v.* controls. Two of the thalassemic children were hepatitis-B surface antigen positive, but none of the controls was positive. In all the thalassemic children serum glucose concentration dropped significantly to < 50 per cent of the fasting level after injecting insulin (0.1 U/kg) intravenously. Thalassemic children had significantly reduced peak GH response to both arginine and clonidine, with significantly lower serum concentration of IGF-I. Basal (8 hour) cortisol, FT4 and TSH concentrations did not differ among the two groups. Serum ferritin concentrations were correlated significantly with peak insulin and glucagon responses to arginine ($r = -0.48$ and $r = 0.42$ respectively, $P < 0.01$) and with IGF-I concentrations ($r = -0.51$, $P < 0.01$) in all the study children.

Discussion

Although the basal serum insulin concentration was normal in thalassemic children after long-term blood transfusion, there was a significant decrease in the maximal insulin secretory capability in response to arginine infusion and oral glucose load indicating a reduced insulin reserve. This gradual and early decrease of insulin secretion in thalassemic children, before the occurrence of impaired glucose tolerance, proves that decreased B-cell mass plays a major role in the development of glucose intolerance later.

In patients with chronic pancreatitis and pancreatic fibrosis the fasting plasma glucose concentration remains normal as long as 20–40 per cent of the B-cell mass is retained. Nonetheless, loss of 20–40 per cent of B-cell mass is associated with a marked impairment in glucose-mediated insulin release, whereas a normal response to arginine is still retained. A further reduction (by 40–60 per cent) in B-cell mass leads to an altered response to arginine. Finally, when the B-cell mass is reduced by more

TABLE 1

Serum glucose, insulin, and glucagon concentrations before and after arginine infusion

		Glucose mg/dl			Insulin ulU/ml			Glucagon pg/ml		
		0-min	30-min	60-min	0-min	30-min	60-min	0-min	30-min	60-min
Controls	Mean	77	89	82	18.6	49.4	23.5	89.6	215	104
$n = 15$	SD	10.2	16.3	12.7	3.3	12.8	5.2	22.8	59.2	36.3
Thalassemia before transfusion	Mean	78.1	97.5	86.6	17.5	41.8	20.9	113.8	247	127.3
$n = 15$	SD	10.8	15.5	17.6	4.7	12.8	5.5	30.7	63.8	45.9
Thalassemia after transfusion	Mean	88.5	109*	91.3	19.3	30.2*†	18.5	157*†	345*†	170*†
$n = 15$	SD	19.7	18.5	21.5	3.6	8.5	8.9	42.5	85.2	53.5

* $P < 0.5$ groups *v.* controls, † $P < 0.05$ before *v.* after prolonged blood transfusion.

Table 2
Serum glucose, insulin, and glucagon concentrations after oral glucose (1.75 g/kg)

		Glucose mg/dl			Insulin ulU/ml			Glucagon pg/ml		
		0-h	1-h	2-h	0-h	1-h	2-h	0-h	1-h	2-h
Controls	Mean	75	87	79.5	17.9	58.1	49.2	84.2	44.9	46.7
$n=15$	SD	9.2	12.2	8.5	5.1	14.2	13.8	21.9	15.6	16.4
Thalassemia before transfusion	Mean	73	92.4	82	19.2	49.7	42.9	99.8	63.2	48.1
$n=15$	SD	10	11.2	7.4	4.3	16.2	11.6	35.6	24.5	11.2
Thalassemia after transfusion	Mean	91	110.8*†	105*†	16.1	36*	31.6*	169*†	146*†	138.5*†
$n=15$	SD	8.9	11.1	9.8	5.8	10.6	9.8	39.9	44.2	38.1

* $P<0.05$ groups *v.* controls; † $P<0.05$ before *v.* after prolonged blood transfusion.

TABLE 3
Growth parameters and hormonal data of thalassemics on blood transfusion

Male/Female		Controls ($n=15$) (10/5)	Thalassemics ($n=15$) (8/7)
Age	Mean	7.3	6.3
(year)	SD	1.8	1.5
B-age-delay	Mean	0.8	−1.5*
(year)	SD	0.6	0.45
HtSDS	Mean	1.1	−1.6*
	SD	0.4	0.5
GV	Mean	6.3	4.1*
(cm/year)	SD	1.2	1.3
BMI	Mean	16.9	13.3*
(kg/m^2)	SD	1.5	1.25
Transf. period	Mean	None	3.1
(year)	SD		0.6
Serum ferritin	Mean	196	1295*
(ng/ml)	SD	54	328
ALT	Mean	18.7	70.2*
(U/l)	SD	11.5	35
Bilirubin	Mean	5.8	11.2*
(μmol/l)	SD	4.5	5.4
Albumin	Mean	39.4	41.2
(g/l)	SD	6.2	5.2
IGF-I	Mean	163.5	82.4*
(ng/ml)	SD	42	18.2
GH-b	Mean	2.1	1.33
(μg/l)	SD	0.8	0.6
GH-p-arg	Mean	15.7	8.2*
μg/l)	SD	2.3	4.7
GH-b	Mean	1.8	1.9
(μg/l)	SD	0.9	1.1
GH-p-Clon	Mean	16.8	7.8*
(μg/l)	SD	3.2	3.8
FT4	Mean	17.5	15.8
(pmol/ml)	SD	2.5	1.5
TSH	Mean	1.5	2.9
(mIU/ml)	SD	0.5	0.77
Cortisol-b	Mean	382	298
(nmol/l)	SD	85	76

B-age = bone age, GH-b = basal GH, GH-p = peak GH, GH-p-arg = GH peak after arginine, GH-p-Clon = GH peak after clonidine, $P<0.05$ thalassemics *v.* controls.

than 80–90 per cent, fasting hyperglycemia and inability to secrete insulin in response to all secretagogues is present.[7,30] It appears that in thalassemic children on frequent and prolonged blood transfusion early iron deposition and gradual fibrosis of the pancreas[1] leads to gradual loss of B-cell mass and impaired response to secretagogues, similar to that of chronic pancreatitis. In addition, impaired insulin secretion in response to both oral glucose and arginine denotes a significant loss of B-cell mass (40–60 per cent) after a mean of 3.1 years of frequent blood transfusion in these patients. Our results support the findings of Dmochowski *et al.*[31] indicating a trend towards progressive reduction in circulating insulin levels in thalassemic patients.

Although the presence of insulin resistance in patients with thalassemia has been suggested by numerous studies,[8,9,15] the finding is not universal. Brianda *et al.*[32] performed euglycemic insulin clamps in thalassemic patients and found increased insulin sensitivity. Our data showed that despite hyperglucagonaemia serum glucose concentrations decreased properly (<50 per cent of the fasting glucose level) after an intravenous insulin (0.1 U/kg) injection in all the thalassemic children ruling out the presence of a significant insulin insensitivity at this age. However, prolonged hyperglucagonaemia and progressive hepatic dysfunction might add an element of insulin resistance at an older age.

In thalassemic children on long-term transfusion the elevated basal serum glucagon concentrations, the significantly higher levels of glucagon and lower insulin/glucagon ratios after provocation with arginine in comparison with controls, and the non-suppressions of circulating glucagon levels after oral glucose load prove a state of hyperglucagonaemia in these children. In other diseases with progressive fibrosis of the pancreas, inflammation and destruction of the parenchyma is associated with collapse between islets leading to formation of clusters of pancreatic islets. Within the remaining islets, a rearrangement of the endocrine cell population occurs with a proportionally greater loss of B-cells compared with A-cells, leading to a reversal of the normal 2:1 ratio.[7] This quantitative change of the islet cells might explain in part the finding of hyperglucagonaemia in our children with thalassemia. In addition, the A-cells are believed to be an insulin-dependent tissue, since inhibition of glucagon secretion by glucose normally occurs in the presence of insulin.[34] In our thalassemic patients impaired insulin secretion in response to glucose load might have contributed to the non-suppression of hyperglucagonaemia. The subnormal GH and cortisol peak responses to provocation exclude any contribution of these anti-insulin hormones in the production of insulin-resistance.

The progressive growth impairment in our thalassemic children might be attributed to their low GH secretion and, consequently, low generation of IGF-I. These data support the view that functional damage in hypothalamic structures for GH control is an important factor contributing to growth delay in these children.[34–36] Although hepatic GH receptor and/or post-receptor defect might explain the low IGF-I generation in thalassemic children with normal GH secretion[34], Postel *et al.*[37], did not find evidence for a defect in GH binding to liver membranes in thalassemic patients, and there was no correlation between the level of GH binding to liver membranes and the degree of hepatic siderosis and fibrosis. Moreover, insulin plays an important role in determining the bioavailability of IGF-I through its action on insulin-like growth factor binding protein-1 (IGFBP-1).[38] Therefore, defective insulin secretion in thalassemic children on long-term blood transfusion might increase hepatic production of IGFBP-1 leading to decreased bioavailability of IGF-I. The low BMI in thalassemic children might be explained in part by the defective secretion of the two major anabolic hormones, namely insulin and IGF-I.

In summary, thalassemic children on long-term high-transfusion and iron chelation develop progressive and early loss of B-cell mass manifested by decreased insulin release in response to secretagogues before impairment of their glucose tolerance. However, hyperglucagonaemia and progressive hepatic dysfunction might add an element of insulin resistance later in life.

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Interleukin-1-beta, Tumour Necrosis Factor-alpha, Islet-cell Antibody, and Insulin Secretion in Children with Thalassemia Major on Long-term Blood Transfusion

by Ahmed El Nawawy, MD, Ashraf T. Soliman, MD, Omar El Azzouni, MD, Amr A. Abbassy, MD, Mohammed N. Massoud, MD, Salah Marzouk,* PhD, *and* Fathia Ibrahim, MD, *and* Laila Helal, MD
*Departments of Pediatrics and *Clinical Pathology, College of Medicine, University of Alexandria, Alexandria, Egypt*

Summary

***In vitro*, cytokines like interleukin-1-beta (IL-1-B) and tumour necrosis factor-alpha (TNF-A) inhibit insulin release and can destroy islet B-cells. We measured blood levels of IL-1-B, TNF-A, and islet cell antibody (ICA) in 20 children with IDDM, 20 of their non-diabetic siblings, 20 children with thalassemia major on long-term hypertransfusion therapy and iron chelation, and 10 normal age-matched children. In the non-diabetic and thalassemic children we investigated the early phase of insulin release after i.v. glucose (0.5 g/kg, 30 per cent solution) and evaluated tolerance to oral glucose (1.75 g/kg). Circulating IL-1-B and TNF-A concentrations were significantly higher in IDDM-siblings (33.7 ± 12.7 pg/ml and 655 ± 165 pg/ml, respectively) *v.* normal children (21.1 ± 6.4 pg/ml and 383 ± 122 pg/ml, respectively). Thalassemic children had no detectable circulating ICA. The prevalence of ICA was 30 per cent in children with IDDM and 60 per cent of their siblings. Impaired oral glucose tolerance was detected in five children with thalassemia (25 per cent), but in none of the IDDM-siblings. The early phase of insulin release was significantly depressed in thalassemic children (peak insulin $= 29.2 \pm 5.1$ mIU/ml) *v.* normal children (52.3 ± 9.5 mIU/ml) and IDDM-siblings (45.3 ± 12.4 mIU/ml). It appears that thalassemic children had significantly decreased insulin secretion and impaired glucose tolerance, however, the mechanism of B-cell dysfunction is not mediated by ICA nor by cytokines.**

Introduction

Despite iron chelation, hypertransfusion regimens lead to chronic iron overload which results in hepatic, cardiac and endocrine dysfunction. Insulin-dependent diabetes (IDDM) and impaired glucose tolerance occurs in a large number of thalassemic patients.[1] Although insulin deficiency secondary to iron deposition has been assumed to be the principle cause of the abnormalities of in glucose metabolism observed in thalassemia,[1–3] other studies reported hyperinsulinaemia suggesting a role of insulin resistance in mediating these metabolic abnormalities.[4,5] However, the early age of onset of diabetes in these patients suggests that an active process contributes to the deterioration of B-cell function.[6]

Recent studies reported increased production of different cytokines in multitransfused thalassemic patients.[7,8] These cytokines might have a role in the pathogenesis of diabetes in thalassemic patients through their cytotoxic effect on islet cells and/or inhibition of insulin release.[9–11]

To clarify this issue we measured the circulating levels of IL-1-B, TNF-A, and islet cell antibody (ICA) in 20 children with IDDM, 20 of their non-diabetic siblings, 20 children with B-thalassemia major, and 10 normal age-matched children. In addition, we investigated the first phase insulin release and oral glucose tolerance (OGT) in the non-diabetic and thalassemic children.

Patients and Methods

Twenty children with IDDM, 20 non-diabetic children randomly selected from their siblings, and 20 children with B-thalassemia major were the subjects of this study. Ten healthy children (mean age 7.8 ± 1.6 years) with no family history of diabetes served as controls. Informed consent was obtained from the parents of all children and, when

Correspondence: Ashraf T. Soliman, MD, Pediatric Endocrinology, Royal Hospital, P.O. Box 1331 Seeb, Muscat, Code 111, Oman.

appropriate, from the children, and the Ethical Committee of the University of Alexandria approved the study. Children were admitted to Alexandria University Children's Hospital for the study. All the children were prepubertal, clinically free from infection, and had no other systemic disease. None of them had history of recent viral infection. All the control children had normal hepatic and renal functions and haemogram. Thalassemic children were on frequent transfusion therapy (15 ml of packed erythrocytes/kg of body weight given every 4 weeks) to keep their haemoglobin at or above 10 g/dl, and iron chelation therapy using i.m. deferoxamine. All had normal thyroid function and none of them had family history of diabetes. None of the diabetics had pancreatic calcification in either plain abdominal X-ray films or by sonographic examination.

A venous blood sample was obtained from all the children for measuring islet cell antibody (ICA), by the indirect immunofluorescent method (INOVA Diagnostics), and IL-1-B and TNF-A using ELISA technique (Medgenix). After 3 days on full carbohydrate diet an oral glucose tolerance test was performed (1.75 g glucose/kg of body wt) and serum glucose measured by glucose oxidase method before and 1 and 2 hours after the oral glucose load for all the non-diabetic children. On the second morning and after an overnight fast a glucose load (0.3 g/kg, 30 per cent solution) was injected i.v. over 2 min and blood collected before and 5 min after the injection for measuring insulin concentration by radio-immunoassay (Diagnostic Product Corporation).

Statistical analysis was carried out using the ANOVA test to compare mean analyte concentrations among the study groups when the data were normally distributed and Wilcoxon test when they were not. Statistical significance was accepted at $P<0.05$.

Results

Table 1 presents the anthropometric and laboratory data of the study groups. IL-1-B and TNF-A concentrations were significantly higher in the IDDM-siblings group *v.* those for the other groups. IL-1-B and TNF-A concentrations did not differ significantly among the thalassemic group, diabetic group, and health controls. Five of the thalassemic children had impaired glucose tolerance after oral glucose load (25 per cent). All the IDDM-siblings had normal oral glucose tolerance. The early phase of insulin release was significantly lower in thalassemic children compared to normal children and IDDM-siblings. ICA was detected in 30 per cent of the diabetic children (group II) *v.* 60 per cent of their

TABLE 1
Anthropometric and lab data of patients and controls

		IDDM ($n=20$)	IDDM-sibs ($n=20$)	Thalassemics ($n=20$)	Controls ($n=10$)
Age (years)	Mean	7.2	8.3	10.6	7.8
	SD	2.9	2.3	2.5	1.6
Weight (kg)	Mean	24.2	26.8	22	24.8
	SD	4.7	13.7	5.2	4.6
Height (cm)	Mean	118.6	124	128.6	120.2
	SD	11.4	17.9	15.2	8.9
ICA (%)	Positive	30*	60*	0	0
	Negative	70	40	100	100
IL-1-B	Mean	15.2	33.7*	14.1	21.2
(pg/ml)	SD	4.6	12.7	6.4	6.4
TNF-A	Mean	299	655*	330	383
(pg/ml)	SD	52.5	165	124	122
Glu (0-min)	Mean	ND	3.9	5.3	3.8
(mmol/l)	SD		1	0.9	0.7
Glu (1-hours)	Mean	ND	6.3	8.3	5.8
(mmol/l)	SD		1.2	1.5	0.5
Glu (2-hours)	Mean	ND	5.8	7.8*	4.7
(mmol/l)	SD		0.8	1.1	0.7
Insulin (b)	Mean	ND	12.5	7.8	8.4
(uIU/ml)	SD		5.3	2.3	3.4
Insulin (p)	Mean	ND	45.3	29.2*	52.3
(mIU/ml)	SD		12.7	5.1	9.5
% increment	Mean	ND	335*	371*	656

Glu = glucose; (b) = basal; (p) = peak; ND = not done; * $P<0.05$.

siblings. None of the thalassemic children had detectable circulating ICA.

Discussion

Cytokines, through modulation of T-cell responses, can induce round cell infiltration (insulinitis)[12] and inhibit insulin release by the B-cells.[10,11] In the IDDM-siblings the significantly high serum levels of IL-1-B and TNF-A, in conjunction with high prevalence of ICA suggested that these cytokines play an important role in the autoimmune aggression against the islet B-cells. Despite their normal OGTT, the percentage increment of their insulin levels after i.v. glucose was significantly lower than the control group. Campbell IL *et al.*[12] suggested that B-cell destruction, in genetically susceptible subject, progresses through stages. Stage 1 is initiated by modification of B-cell either by viral, chemical, or environmental factor. This leads to hyperexpression of Class-I major histocompatibility complex (MHC) molecules and induction of MHC class II molecules. Stage II commences with infiltration of islets by immuno-inflammatory cells (insulinitis) and production of cytokines from infiltrating cells. This induces 'phenotypic switching' of islet cells. Stage III encompasses autoimmune-mediated destruction of B-cells by the targeted delivery of cytotoxic cytokines and other mediators. The normal serum levels of cytokines in children with IDDM can be explained by the fact that cytokine production is suppressed after extensive destruction of the target cells (B-cells).

In thalassemic children on frequent blood transfusion, the initiation of immune reaction against the B-cells, by the excessive iron deposition and/or by the foreign cellular elements in the transfused blood, constitutes a possible pathogenic factor. However, the normal serum concentrations of IL-1-B and TNF-A and the absence of circulating ICA in thalassemic children despite their high prevalence of impaired glucose tolerance and decreased insulin response to i.v. glucose, exclude an important role, if any, played by the immune system in the aetiology of diabetes in these children.

In summary, it appears that over time children with thalassemia experience a reduction in their circulating insulin levels which leads to glucose intolerance and diabetes mellitus. This B-cell destruction is mediated neither by ICA nor by cytokine production.

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European Journal of Endocrinology (1998) 263 000–000 ISSN 0804-4643

GH response to provocation and circulating IGF-I and IGF-binding protein-3 concentrations, the IGF-I generation test and clinical response to GH therapy in children with β-thalassaemia

Ashraf T Soliman, Nagwa El Banna and B M Ansari[1]

Department of Paediatrics, University of Alexandria School of Medicine, Alexandria, Egypt, and [1]Department of Paediatrics, University of Glamorgan School of Medicine and East-Glamorgan General Hospital and School of Applied Sciences, University of Glamorgan, Pontypridd, Wales, UK

(Correspondence should be addressed to A T Soliman, Alexandria University Children's Hospital, El-Chatby, Alexandria, Egypt)

Abstract

The causes of growth retardation of children with thalassaemia major are multifactorial. We studied the GH response to provocation by clonidine and glucagon, measured the circulating concentrations of insulin, IGF-I, IGF-binding protein-3 (IGFBP-3) and ferritin, and evaluated IGF-I generation after a single dose of GH (0.1 mg/kg per dose) in 15 prepubertal patients with thalassaemia, 15 age-matched children with constitutional short stature (CSS) (height standard deviation score less than −2, with normal GH response to provocation) and 11 children with isolated GH deficiency (GHD). Children with thalassaemia had significantly lower peak GH response to provocation by clonidine and glucagon (6.2 ± 2.3 and 6.8 ± 2.1 μg/l respectively) than the CSS group (18.6 ± 2.7 and 16.7 ± 3.7 μg/l respectively). They had significantly decreased circulating concentrations of IGF-I and IGFBP-3 (49.7 ± 19 ng/ml and 1.2 ± 0.27 mg/l respectively) compared with those with CSS (153 ± 42 ng/ml and 2.06 ± 0.37 mg/l respectively), but the IGF-I and IGFBP-3 concentrations were not different from those with GHD (56 ± 25 ng/ml and 1.1 ± 0.32 mg/l respectively). These data demonstrate that the GH–IGF-I–IGFBP-3 axis in thalassaemic children is defective. Serum ferritin concentration correlated significantly with GH peak response to provocation ($r = -0.36$, $P < 0.05$) and circulating IGF-I ($r = -0.47$, $P < 0.01$) and IGFBP-3 ($r = -0.42$, $P < 0.01$) concentrations. In the IGF-I generation test, after GH injection, the thalassaemic children had significantly lower IGF-I and IGFBP-3 levels 86.7 ± 11.2 ng/ml and 2.07 ± 0.45 mg/l respectively) than those in the CSS group (226 ± 45.4 ng/ml and 2.8 ± 0.43 mg/l respectively). The IGF-I response was significantly higher in children with GHD (158 ± 50 ng/ml) than in thalassaemic children. Six short (height standard deviation score less than −2) thalassaemic children who had defective GH response to provocation (<10 μg/l), all the children with GHD and eight short normal children (CSS) were treated for 1 year with human GH (18 units/m^2 per week divided into daily s.c. doses). After 1 year of GH therapy there was a marked acceleration of growth velocity in both thalassaemic children (from 3.8 ± 0.6 cm/year to 7.2 ± 0.8 cm/year) and controls. However, the linear acceleration of growth velocity on GH therapy was significantly slower in thalassaemic children (3.3 ± 0.3 cm/year increment) compared with those with CSS (5.3 ± 0.4 cm/year increment) and GHD (6.9 ± 1.2 cm/year increment) ($P < 0.05$). Their circulating IGF-I concentration (105 ± 36 ng/ml) was significantly lower than those for CSS (246 ± 58 ng/ml) and GHD (189 ± 52 ng/ml) after 1 year of GH therapy. These data prove that some children with β-thalassaemia major have a defective GH–IGF-I–IGFBP-3 axis and suggest the presence of partial resistance to GH.

European Journal of Endocrinology 263 000–000

Introduction

Growth and maturational delay are striking features of β-thalassaemia major. After the age of 4 years, significant growth retardation involves stature, sitting height, weight and skeletal maturation (1–3). Delayed or complete lack of pubescent changes are common in both girls and boys (4, 5). Haemosiderosis-induced damage of the endocrine glands is implicated as one of the main causes for the growth failure (6).

Basal circulating concentrations of various hormones have been studied by different authors with no consensus defining the different endocrine abnormalities of the growth hormone (GH)–insulin-like growth factor-I (IGF-I)–IGF-binding protein-3 (IGFBP-3) axis. Both normal (7, 8) and subnormal GH response (9, 10) to

provocative stimulation tests have been reported. Some of those with normal GH response to provocation have neurosecretory dysfunction of GH secretion (9, 11, 12).

IGF-I is a GH-dependent polypeptide that has a 3-fold function as a mediator of the growth-promoting action of GH, as a potent mitogenic factor, and as a metabolic regulator with insulin-like activity (13, 14). Alterations in IGF-I regulation may provide an attractive explanation for thalassaemia-associated growth impairment. The predominant IGFBP in blood is IGFBP-3, which forms a large 150 kDa ternary complex. The serum level of this complex determines the total concentration of circulating IGF-I and regulates its growth-promoting potential (15, 16). Current opinion favours GH as the major regulator of IGF-I and IGFBP-3 levels in humans. In addition, serum IGF-I and IGFBP-3 are positively related to nutritional status (17) and affected by other hormones such as insulin (18). Estimation of circulating concentrations of IGF-I and IGFBP-3 markedly improves the interpretation of GH data in response to provocation and allows better evaluation of the hypothalamic–pituitary growth axis.

This study was conducted to (1) investigate the GH–IGF-I–IGFBP-3 axis in prepubertal children with β-thalassaemia major, (2) test the hypothesis that these patients might have GH resistance, and (3) study the effect of GH therapy for 1 year on their growth parameters.

Patients and methods

Fifteen prepubertal children, between the age of 7.5 and 14 years, with β-thalassaemia major were randomly recruited for the study from the thalassaemia clinic of the Alexandria University Children's Hospital, Alexandria, Egypt. All had been treated with a chronic low-transfusion regimen (to keep haemoglobin concentration above 8 g/dl), with i.m. administered chelation treatment (3 times per week; suboptimal chelation for economic reasons) and were taking 5 mg folic acid per day. Fifteen prepubertal age-matched children with constitutional short stature (CSS), age range 7–13 years (with height standard deviation scores (HtSDS) at or below −2 and normal GH response to provocation) and 11 children with isolated GH deficiency (GHD), age range 6.5–10.5 years (GH peak response <5 μg/l in two or more provocative tests) served as controls. Informed consent was obtained from the parents of all the patients and, when appropriate, from the children before including them in the study. The ethical committee of Alexandria University approved the protocol of the study. None of the children had intrauterine growth retardation, severe malnutrition, diabetes, dysmorphic traits, exposure to irradiation, or any other systemic illness. All were prepubertal, with pubarche Tanner 2 or less, gonadarche stage in the boys of 1 and larche stage in the girls of 1, and all were euthyroid.

Anthropometric measurements included weight (obtained to the nearest 100 g using a digital scale (Seca model 770)), height (using the Harpenden scale), mid-arm circumference (using a metal tape) and triceps skinfold thickness (using the Holtain calliper). Growth velocity (cm/year) was measured over a whole year for all the patients before the start of any GH therapy. HtSDS and body mass index (BMI) were calculated. Normal population data were those reported by Tanner & Whitehouse (19). Nutritional assessment included evaluation of dietary intake using the recall method for the preceding 3 days. These data were recorded every clinic visit for the whole year (minimum of three visits a year).

After an overnight fast, a venous sample was withdrawn for measurement of free thyroxine (FT_4), thyrotrophin, IGF-I, IGFBP-3 and 0800 h cortisol concentrations. Serum ferritin, albumin, globulin, bilirubin, alanine aminotransferase, creatinine, calcium, phosphate and alkaline phosphatase were measured. Cortisol concentration was measured 1 h after i.v. injection of adrenocorticotrophin (ACTH; 0.5 mg Synacthen). Two GH provocative testings with clonidine (0.15 mg/m^2 orally) and glucagon (0.1 mg/kg i.m.) were performed on two occasions. After 3 days of adequate carbohydrate intake, a standard oral glucose tolerance test (1.75 g/kg glucose) was carried out in thalassaemic children. The IGF-I generation test (20) was performed in all the patients. The test entails measurement of morning basal circulating IGF-I concentration, followed by injection of human GH (0.1 mg/kg per dose, s.c.) and remeasuring IGF-I concentration next morning.

Six patients with β-thalassaemia major with growth retardation (HtSDS and growth velocity standard deviation score at or below −2 for their chronological age and for their respective bone age, determined by the method of Greulich and Pyle) and defective GH release in two provocative tests were treated with human GH (18 units/m^2 per week divided into daily s.c. doses) for 1 year. Growth parameters were followed-up every 3 months for the whole year, and IGF-I concentrations remeasured at the end of the year. Children with GHD ($n = 11$) and those with CSS ($n = 8$) treated with GH (18 units/m^2 per week divided into daily doses) for the same period were used as controls. The oral glucose tolerance test was performed every 6 months in the GH treatment groups.

Human GH and IGF-I were measured by radioimmunometric assay, employing reagents purchased from Nichols Institute (San Juan Capistrano, CA, USA). Intra-assay coefficients of variation (CVs) averaged 5.8 and 6.6% respectively, and interassay CVs averaged 7.6 and 8.4% respectively in the range of GH and IGF-I values detected. IGFBP-3 was measured by RIA by SCL Bioscience Services employing reagents supplied by Mediagnost. The assay sensitivity was 0.06 μg/ml with intra- and inter-assay CVs of 5.2 and 8.6% respectively.

Data are presented as mean ± s.d. Statistical analyses were performed using the ANOVA test to compare analyte concentrations among groups. The paired *t*-test was used to compare data before and after therapy in the same group. Wilcoxon test was used when the data were not normally distributed. Correlations between variables of interest were examined by linear regression analysis and, when appropriate, multiple regression analysis.

Results

Table 1 summarises the auxologic data of prepubertal children with thalassaemia, GHD and CSS. The HtSDS was significantly lower in children with GHD vs children with CSS and thalassaemia. Linear growth velocity and BMI did not differ significantly among the three groups studied. The bone age was significantly delayed in the GHD group. The upper/lower segment ratio was significantly lower in thalassaemic children than in the other two groups denoting slower growth of the spine compared with the limbs in this group of patients.

The biochemical and hormonal data (Table 2) show that thalassaemic children had significantly higher concentrations of serum ferritin and bilirubin and alanine aminotransferase activity, and lower haemoglobin and haematocrit values than those with CSS. All the children had normal serum creatinine and albumin concentrations. Two children with thalassaemia had impaired tolerance to oral glucose (1.75 g/kg) and were excluded from the GH treatment group. Serum calcium and phosphorus concentrations and alkaline phosphatase activity were comparable with those for controls, ruling out the diagnosis of hypoparathyroidism in any of the patients. After ACTH stimulation, circulating cortisol concentrations were significantly lower in thalassaemic children than controls. In three thalassaemic children, cortisol concentrations did not rise to 400 nmol/l. However, none of them had symptoms or signs of cortisol deficiency. Thyroid function was normal in 14 of 15 children with thalassaemia. One patient had mild hypothyroidism. His FT_4 was 9 pmol/ml (normal 10–25 pmol/l) and elevated TSH of 8 μU/ml (normal 0.5–5 μU/ml). GH testing was performed after T_4 replacement for 1 month in this boy. Fasting serum insulin concentrations did not differ between thalassaemic children (12 ± 7.8 μg/l) and controls (14.5 ± 5.8 μg/l).

GH/IGF-I/IGFBP-3 data are presented in Table 3. Thalassaemic children had significantly lower peak GH response to provocation by clonidine and glucagon than the childre with CSS. Their circulating IGF-I and IGFBP-3 concentrations were significantly lower than those for the controls with CSS. The IGF-I and IGFBP-3 responses to GH injection were significantly lower in thalassaemic patients than controls, suggesting partial resistance to GH in these children. Their serum IGF-I concentrations after stimulation with GH were still lower than the basal circulating levels for the controls.

Table 3 shows the GH, IGF-I and IGFBP-3 data of all the patients. The peak GH response to provocation and circulating IGF-I concentrations was significantly lower in thalassaemic children than in those with CSS ($P < 0.005$). The IGF-I response to GH administration (IGF-I D in Table 3) (equal to the 24 h IGF-I value minus the basal value) was significantly lower in children with thalassaemia than in those with CSS or GHD ($P < 0.01$).

Table 4 compares growth and IGF-I data of six thalassaemic children with those for eight children with CSS and 11 children with GHD. After 1 year of GH therapy (18 units/m^2 per week divided into daily s.c. doses) the growth velocity of patients with thalassaemia (7.2 ± 0.8 cm/year) was slower than that for children with CSS (9.9 ± 1.2 cm/year). The increments of growth velocity per year of thalassaemic patients was significantly lower than for the other two groups. Despite slower growth in thalassaemic children, their growth velocity doubled (from 3.8 ± 0.6 cm/year to 7.2 ± 0.8 cm/year). After GH therapy for 1 year the circulating IGF-I concentrations were significantly lower in thalassaemic patients than controls. None of the children developed impaired glucose tolerance or hypertension during treatment.

Correlations between circulating hormonal and ferritin concentrations for all the children are presented in Table 5. Serum ferritin concentration correlated significantly (negatively) with GH peak response, IGF-I, IGFBP-3 and insulin concentration. Peak GH response correlated significantly with IGF-I and IGFBP-3, supporting the view that GH is the major regulator of both IGF-I and IGFBP-3 synthesis.

Table 1 Auxologic data of patients and controls. Values are mean ± s.d.

	No	Sex (M/F)	Age (years)	Bone age delay (years)	HtSDS-1	HtSDS-2	Upper/lower segment	Growth Velocity (cm/year)	BMI (kg/m^2)
Thalaassaemia	15	9/6	7.9 ± 1.8	1.7 ± 0.4	1.9 ± 0.55	2.04 ± 0.5	0.85 ± 0.05	4 ± 1.3	14.8 ± 1.1
CSS	15	8/7	8.1 ± 0.8	2.5 ± 0.8	2.3 ± 0.3	2.2 ± 0.5	1.1 ± 0.07	4.6 ± 0.6	13.8 ± 0.6
GHD	11	6/5	7.3 ± 1.8	2.8 ± 0.4	3.1 ± 0.58*	3.4 ± 0.5*	1.18 ± 0.08	3.7 ± 0.6	15.5 ± 1.2

HtSDS-1 and -2 means before and after 1 year of GH therapy.
*$P < 0.05$ among groups.

Table 2 Biochemical and hormonal data. Values are mean ± s.d. ($n = 15$).

	Ferritin (ng/ml)	Haemoglobin (g/dl)	ALT (U/l)	Bilirubin (μmol/l)	Albumin (g/l)	Calcium (mmol/l)	Phosphate (mmol/l)	ALP (U/l)	FT_4 (pmol/ml)	Thyrotrophin (μU/ml)	Cortisol (nmol/l)	
											1	2
Thalassaemia	1388 ± 411	7.8 ± 1.3*	78 ± 38*	24 ± 6*	41 ± 5.4	2.1 ± 0.02	1.4 ± 0.03	188 ± 54	15.7 ± 3.4	3.2 ± 0.8	311 ± 78	432 ± 172*
CSS	109 ± 54	13.5 ± 1.1	18.7 ± 11.5	5.8 ± 4.5	39 ± 6.2	2.2 ± 0.03	1.5 ± 0.08	184 ± 49	17.5 ± 2.9	1.5 ± 0.5	382 ± 85	688 ± 188

1 and 2 mean cortisol concentration before and after ACTH administration.
ALT, Alanine aminotransferase; ALP, alkaline phosphatase.
* $P < 0.05$ compared with values for CSS group.

Discussion

The growth-promoting activity of IGF-I is determined not only by the concentration of IGF-I but also by the amounts of various IGFBPs (21, 22). Of these, IGFBP-3 is the major binding protein (23). It has been observed to potentiate the effects of IGF-I in bone (24–26). In addition, recent data clearly indicate that IGFBP-3 prolongs the half-life of circulating IGF-I levels and changes the clearance pattern of plasma IGF-I (27). Current opinion favours GH as the major regulator of IGF-I and IGFBP-3 in humans. In this study, the GH response to provocation with clonidine and glucagon was impaired in short prepubertal children with β-thalassaemia major compared with that in short normal children. Ten of the 15 children with thalassaemia did not mount an appropriate GH response (>10 μg/l) in both provocation tests. These findings support previous reports indicating impairment of function along the hypothalamic–pituitary growth axis (9–12, 28). In malnourished children with decreased IGF-I synthesis, the basal and stimulated GH levels are significantly higher than normal (29), indicating stimulation of their hypothalamic–pituitary axis by the low circulating concentrations of IGF-I. In thalassaemic children, the presence of normal basal GH levels despite low circulating IGF-I levels suggests a defective hypothalamic–pituitary feedback mechanism. This might be secondary to defective GH secretion. Histopathological changes with significant siderosis of the pituitary gland and secondary atrophy of the somatotrophs can explain the dysfunction of this axis (6) and the increased incidence of defective GH secretion in our children, with defective chelation therapy, compared with other studies with properly chelated patients. Impaired GH secretion can explain in part the significantly lower IGF-I and IGFBP-3 synthesis with subsequent growth impairment in children with thalassaemia major. However, haemosiderosis of the liver in these patients with disturbed hepatic function may also decrease IGF-I synthesis. In our study serum ferritin concentration correlated significantly with IGF-I concentration ($r = -0.45$, $P < 0.05$), IGFBP-3 ($r = -0.42$, $P < 0.05$) and peak GH response to provocation ($r = -0.34$, $P < 0.05$), which supports the view that iron overload may affect GH/IGF-I/IGFBP-3 secretion adversely. The deficiency of IGFBP-3 in thalassaemic patients may contribute to their growth impairment by decreasing the growth-promoting effects of IGF-I. We (30) and others (31) reported progressive impairment of insulin secretion in children with thalassaemia because of haemosiderosis of the pancreas. Other investigators stressed as well the importance of insulin resistance in these patients (31, 32). The progressive loss of the anabolic functions of insulin may contribute to the delayed growth of these children either directly and/or through inhibition of IGF-I synthesis and function (18, 33). In our study basal (fasting) serum concentrations

Table 3 Responses of IGF-1 and IGFBP-3 to GH. Values are mean ± S.D.

	Thalassaemia ($n = 15$)	CSS ($n = 15$)	GHD ($n = 11$)
IGF-I (ng/ml) before GH	47.5 ± 19	153 ± 42*	56 ± 25
IGF-I (ng/ml) after GH	86.7 ± 11.2	226 ± 45.4*	158 ± 50
IGF-I (ng/ml) D	38.2 ± 11.8	73 ± 11.7	95 ± 24*
IGFBP-3 (mg/l) before GH	1.2 ± 0.27	2.06 ± 0.37*	1.1 ± 0.32
IGFBP-3 (mg/l) after GH	2.05 ± 0.51	2.8 ± 0.43*	ND
IGFBP-3 (mg/l) D	0.8 ± 0.36	0.71 ± 0.35	ND
Peak GH response to clonidine (μg/l)	6.2 ± 2.3	18.6 ± 2.7*	4.2 ± 0.9
Peak GH response to glucagon (μg/l)	6.8 ± 2.1	16.7 ± 3.7*	3.9 ± 1.5

D means the response of IGF-I or IGFBP-3 above basal levels after GH injection. ND, not determined.
* $P < 0.05$ compared with values for thalassaemic children.

of insulin correlated significantly with concentrations of IGF-I ($r = 0.541$, $P < 0.01$) and IGFBP-3 ($r = 0.42$, $P < 0.05$). Delayed or arrested puberty is common in patients with thalassaemia (4, 5) because of disturbed gonadotrophin-releasing hormone secretion (5, 34) with consequent deficiency of sex steroids. Sex steroids can influence growth through the modulation of IGF-I-induced cellular response (35–37), and their deficiency adds significantly to the growth delay and osteoporosis of thalassaemic children (38). This may explain the relatively short upper segment, in addition to the mild vertebral changes observed in our thalassaemic group.

Malnutrition, primarily caused by inadequate nutrient intake, as indicated by the capacity to gain weight appropriately when provided with nutritional support (39), is another correctable cause of growth delay in thalassaemic children. Malnutrition can inhibit growth through inhibition of IGF-I (29) and IGFBP-3 (17) synthesis and insulin release (29). However, our group of patients had normal BMI, mid-arm circumference and skinfold thickness, and normal serum albumin and basal GH concentrations. Analysis of their dietary intake, by using the recall method, showed normal quantitative and qualitative dietary intake. These factors collectively exclude any major role played by malnutrition in our children with thalassaemia.

Impaired linear growth in thalassaemic children, despite regular transfusion and desferroxamine therapy, who have normal GH secretion suggests the possibility of GH resistance. In this study the IGF-I generation test showed that these patients do not secrete adequate IGF-I after GH stimulation when compared with normal short children or those with GHD. After 1 year of GH therapy, despite the same dose/m^2 being given to all the children, their circulating IGF-I concentrations were significantly lower than those for the short normal control group and those with GHD. These data are in concert with those of Werther *et al.* (40), who reported lack of response of non-suppressible insulin-like activity to short-term administration of human GH in their thalassaemic patients. In our six thalassaemic children treated with GH, the growth velocity increased significantly from 3.8 ± 0.6 cm/year to 7.2 ± 0.8 cm/year (doubling). However, the increment in the rate of growth was significantly smaller than that of the control groups. Collectively these findings may suggest the presence of significant GH resistance in these patients, which could attenuate the growth-promoting effects of GH therapy. Low *et al.* (41) showed that, with higher (supraphysiological) (30 units/m^2 per week divided into daily s.c. doses) doses of exogenous GH, there was a progressive increase in IGF-I production in their thalassaemic patients. It is known that higher GH doses during treatment possibly elicit a higher IGF-I and growth velocity response; however, this high dose may be necessary in thalassaemic children to overcome the possible GH resistance. However, supraphysiological doses of GH may increase the risk of inducing diabetes (30–32) and hypertension (42) in these high-risk patients. Human IGF-I therapy, alone or in combination

Table 4 Auxological and IGF-1 data before and after GH therapy. Values are mean ± S.D.

		Growth velocity (cm/year)			HtSDS		IGF-I (ng/ml)	
	Age (years)	1	2	Increment	1	2	1	2
Thalassaemia ($n = 6$)	6.2 ± 1.5	3.8 ± 0.6	7.2 ± 0.8*	3.3 ± 0.3	2.1 ± 0.3	1.7 ± 0.3	56.3 ± 23.5	105 ± 36*
CSS ($n = 8$)	6.8 ± 1.7	4.5 ± 0.4	9.9 ± 1.2*†	5.3 ± 0.4†	2.6 ± 0.4	1.8 ± 0.42*	142 ± 49†	246 ± 58*†
GHD ($n = 11$)	7.3 ± 1.8	3.7 ± 1.0	10.6 ± 1.5*†	6.9 ± 1.2†	3.4 ± 0.5†	2.6 ± 0.4†	66 ± 25	189 ± 59*†

1 and 2, before and after 1 year of GH therapy.
* $P < 0.05$ vs before GH therapy. † $P < 0.05$ vs values for thalassaemic children.

Table 5 Correlation between hormonal and ferritin values (*r* values)

	Peak GH	IGF-I	IGFBP-3	Insulin
Ferritin	−0.36	−0.47	−0.42	−0.41
IGF-I	0.63	1.00	0.71	0.51
IGFBP-3	0.49	0.7	1.00	0.38

with GH and/or IGFBP-3, appears to be an attractive alternative to be used to overcome the GH resistance and avoid the high risk of developing diabetes. In human (43, 44) and animal (26, 27) experiments this combination of growth factors appears to be useful.

In summary, children with β-thalassaemia and short stature have a defective GH–IGF-I–IGFBP-3 axis that might be secondary to haemosiderosis of the pituitary gland, liver and pancreas. In addition to regular blood transfusion and proper chelation therapy, these patients need early management of their endocrinopathy. Treatment of their hypothyroidism, hypogonadotropic hypogonadism, diabetes mellitus and defective GH–IGF-I–IGFBP-3 axis can markedly improve their growth. In addition, these patients may have partial GH resistance which requires supraphysiological doses of GH and/or human IGF-I therapy.

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Received 26 March 1997
Accepted 24 October 1997

SPONTANEOUS AND PROVOKED GROWTH HORMONE (GH) SECRETION AND INSULIN-LIKE GROWTH FACTOR-I (IGF-I) AND IGF-BINDING PROTEIN-3 (IGFBP3) CONCENTRATIONS IN PATIENTS WITH BETA-THALASSEMIA AND DELAYED GROWTH

By:
Ashraf T Soliman MD
Mahmoud M ElZalabany MD
Said M Aziz Bedair MD
Yasser Mazloum MD
Mahmoud Gaber PhD
Alan D Rogol MD, PhD*
B M Ansari FRCP**

The Departments of Paediatrics
The Universities of Alexandria , Virginia* and Glamorgan**
Schools of Medicine, Alexandria, Egypt, Charlottesville VA and
East-Glamorgan General Hospital, and School of Applied Sciences,
University of Glamorgan, Pontypridd, Wales, UK*

Corresponding Author:

Dr. Ashraf T. Soliman MD
Associate Professor of Paediatrics
Alexandria University Children's Hospital
El-Chatby, Alexandria, Egypt.
Phone: 00203-859076, Fax 00203-5876828

ABSTRACT

The growth retardation of children with thalassaemia major is multifactorial. We studied the growth hormone (GH) response to provocation by clonidine and glucagon, measured the circulating concentrations of insulin, insulin-like growth factor-I (IGF-I), IGF-binding protein-3 (IGFBP3), and ferritin, and evaluated the spontaneous nocturnal (12-h) GH secretion in prepubertal patients with thalassaemia and age-matched children with constitutional short stature (CSS) (height SDS < -2, with normal GH response to provocation). The hypothalamic pituitary area was studied in patients with anormal GH secretion using MRI scanning. Children with thalassaemia had significantly lower peak GH response to provocation by clonidine and glucagon (8.8 +/- 2.3 μ/L and 8.2 +/- 3.1 μg/L respectively) vs controls (17.6 +/- 2.7 ug/L and 15.7 +/- 3.7 ug/L respectively). They had significantly decreased circulating concentrations of IGF-I and IGFBP3 (68.5 +/- 19 ng/ml and 1.22 +/- 0.27 mg/L respectively) compared to controls (153 +/- 42 ng/ml and 2.16 +/- 0.37 mg/L respectively). Seven of the thalassemic children had GH peak response < 7μg/L after provocation. Thalassemic patients with normal GH response after provocation, also had significantly lower IGF-I and IGFBP3 concentrations compared to controls. Analysis of their GH pulse properties revealed lower mean (2.9 +/-1.77μg/L) and integrated (2.53 +/- 1.6 μg/L) concentrations versus controls (4.9 +/- 0.29 μg/L and 5.6 +/- 0.52 μg/L respectively). They also had lower mean pulse amplitude (9.2 +/- 2.2 μg/L) versus controls (17.2 +/- 2 μg/L). Five of them had mean nocturnal GH concentration < 2μg/L and four had maximum nocturnal peak below 10 μg/L. These data denoted neurosecretory dysfunction of GH secretion in some of these patients. MRI studies revealed complete empty sella (n= 2) , marked diminution of the pituitary size (n=4). focal hypo/isointense lesions within anterior lobe pituitary gland at T1 and markedly hypointense at T2 (n=4) with variable sized focal hypo/isointense lesions within the midbrain at T1 and markedly hypointense at T2 (n=6). These findings are Consistent with *heamosedrin* deposition in the anterior pituitary and mid-brain of thalassaemic patients with defective GH secretion. Serum ferritin concentration was correlated significantly with the circulating IGF-I ($r = -0.47$, $P < 0.01$), and IGFBP3 ($r = -0.43$, $p < 0.01$) concentrations. These data prove high incidence of functional and structural abnormalities of the GH/IGF-I/IGFBP3 axis in short children with thalassemia major manifested by either defective GH response to provocation and/or neurosecretory dysfunction of GH secretion. However, partial resistance to GH can not be excluded in some of these patients.

INTRODUCTION

Growth and maturational delay are striking features of beta thalassaemia major. After the age o years significant growth retardation involves stature, sitting height, weight as well as skeletal maturation.(1-3) Delayed or complete lack of pubescent changes are common in both girls and boys.(4,5) Haemosiderosis-induced damage of the endocrine glands is implicated to be one of the main causes for their growth failure. (6)

The basal circulating concentrations of different hormones have been studied by different author with no consensus defining the different abnormalities of their growth hormone (GH) / insulin-lil growth factor-I (IGF-I) / IGF-binding protein-3 Axis. Both normal (7,8) and subnormal GH response (9,10) to provocative stimulation test have been reported. Some of those with normal GI response to provocation might have neurosecretory dysfunction of GH secretion. (9,11,12)

IGF-I is a GH- dependent polypeptide that has a 3-fold function as a mediator of the growth promoting action of GH, as a potent mitogenic factor, and as a metabolic regulator with insulin-like activity. (13,14) Alteration of IGF-I regulation may provide an attractive explanation for thalassaemia -associated growth impairment. The predominant insulin-like growth factor bindin proteins IGFBPs in the blood is IGFBP3, which forms a large 150-kilodalton ternary complex. The serum level of this complex determines the total concentration of circulating IGF-I and regulates its growth promoting potential. (15,16) Current opinion favours GH as the major regulator of IGF-I and IGFBP3 levels in humans. In addition, serum IGF-I and IGFBP3 are positively related to nutritional status (17) and affected by other hormones like insulin (18). Estimation of circulating concentrations of IGF-I and IGFBP3 markedly improves the interpretation of GH data in response to provocation and allows better evaluation of the hypothalamic-pituitary growth axis.

This study was conducted to 1. investigate the GH/IGF-I/IGFBP3 axis in short prepubertal children with beta thalassaemia major and 2. measure the pituitary size by MRI in these children

PATIENTS AND METHODS

Seventeen children with β thalassaemia major and delayed puberty were recruited for the study from the thalassaemia clinic of the Alexandria University Children's Hospital, Alexandria Egypt for the study. All have been treated with a chronic low transfusion regimen (to keep their haemoglobin above 9g/dl) , with IM chelation treatment (3-5 times/ week) (suboptimal chelation due to economic reasons) and were taking 5 mg of folic acid per day. Fifteen prepubertal age-matched children with constitutional short stature (CSS) (with height standard deviation scores at or below -2 and normal GH response to provocation) served as controls. Criteria for entry into the study were as follows: 1. Age> 13 and < 25 years; 2. height less than the fifth percentile; 3. Delayed puberty , defined as pubarche Tanner 2 or less, gonadarche stage in the boys of 1 and thelarche stage in the girls of 1, at the age of 14 year and 13 year respectively, or slow progression of the puberty was diagnosed in 12 out of the 17 thalassemic patients. None of the children had intrauterine growth retardation, severe malnutrition, diabetes, dysmorphic trait, exposure to irradiation, or any other systemic illness. Preliminary investigations included measurement of electrolytes, serum calcium, phosphate, alkaline phosphatase, ferritin, urea, and creatinine concentrations, urinalysis and complete blood cell count. Skeletal maturation was determined by the method of Greulich and Pyle.(19) Informed consent was obtained from the patients and their parents before including in the study. The ethic Committee of Alexandria University has approved the protocol of the study.

Anthropometric measurements included weight (obtained to the nearest 100 g using a digital scale (Seca model 770), height (using the Harpenden scale), mid-arm circumference (using a metal tape), and triceps-skinfold thickness (using the Holtain Calliper). Growth velocity cm/yr was measured over a whole year for all the patients. Height standard deviation score (HtSDS) and body mass index (BMI) were calculated. Normal population data were according to Tanner et al . (20)

After an overnight fast a venous sample was withdrawn for measurement of free thyroxine (FT4) , thyrotropin (TSH), insulin-like growth factor-I, IGFBP3 , and 8:00 A.M. cortisol concentrations. Two GH provocative testings with Clonidine (0.15 mg/m2 orally) and glucagon (0.1 mg/kg I.M.) were performed on two occasions. Thalassemic children with normal GH response to provocation (peak GH = or > 10 UG/L) (n = 10) underwent a 12-hour study of spontaneous GH secretion starting at 8 p.m. An indwelling catheter was inserted in a forearm vein, and a continuous blood sample was obtained in 20-min fractions through a peristaltic pump (Cormed, Medina, NY). GH peak analysis was performed visually by examining a plot of serum GH against time. For each GH profile the integrated concentration, the mean pulse amplitude, and the number of peaks above 5 ng/ml were analysed. Three randomly selected children with CSS, matched for chronological age, underwent similar 12-hour profiles of GH.

Human GH and IGF-I were measured by radioimmunometric assay, employing reagents purchased from Nichols Institute (San Juan Capistrano, CA). Intra-assay coefficients of variations (CVs) averaged 5.8% and 6.6% respectively, and interassay CVs averaged 7.6% and 8.4% respectively in the range of GH and IGF-I values detected. IGFBP3 was measured by radioimmunoassay in Serono Bioscientific Laboratories and Bioscience Services employing reagents supplied by Mediagnost. The assay sensitivity = 0.06 ug/ml with intra and inter CVs of 5.2% and 8.6% respectively.

MRI study of the hypothalamic-pituitary area was performed in 10 patients with thalassemia (five with GH deficiency, two with abnormal spontaneous nocturnal GH release and 3 with normal GH secretion), using a gyroscan Philips 1.5.T (Tesla) machine performing the following sequences : -1. Sagittal T1,wI , 2. Coronal T1,wI for the sella tursica , 3. Axial T1 wI for the whole brain, 4. Axial T2 wI over the pituitary and hypothalamic regions 5. Sagittal T2 wI for the brain. The field of view ranged between 180 mm for the pituitary region to 220 mm for the whole brain. Slice thickness was 3 and 6 mm and interslice gap = 0.3 and 0.6 mm for the pituitary and the whole brain respectively. Data are presented as mean +/- SD. A control group consisted of 10 aged between 14 and 23 years with HtSDS = 0.5+/- 0.3 were used as controls for the evaluation of pituitary size (5 males and 5 females). None of them had received cranial radiation therapy and they had all been referred for MRI for various neurological reasons.

Statistical analyses were performed using the unpaired t test to compare analyte concentrations among groups. Wilcoxon test was used when the data were not normally distributed. Correlations between variables of interest are examined by linear regression analysis .

RESULTS

Table (1) summarises the auxologic data of thalassemic patients and controls. The HtSDS, BMI, and GV did not differ significantly among the two groups.

The biochemical and hormonal data (Tables 2,3) showed that thalassemic children had significantly higher concentrations of serum ferritin, ALT and bilirubin and lower hemoglobin values versus the control group. Children of both groups had normal serum T4, TSH , 8:00 A.M. cortisol, creatinine, albumin, calcium, phosphate and ALP concentrations. The circulating concentrations of IGF-I and IGFBP3 were significantly decreased in thalassemic patients versus controls (table 3). The peak GH response to provocation by clonidine and glucagon was significantly low in thalassemic patients. Seven , out of the 17, patients with thalassemia had classic GH deficiency (peak GH < 7 μg/L after provocation by clonidine and glucagon, with low IGF-I , and IGFBP3 concentrations).

These 10 patients underwent 12-h study of nocturnal GH release (figure 1). Analysis of the pulsatile properties revealed that the integrated and mean GH concentrations over 12h were markedly lower in thalassemic patients versus controls . Five out of the ten studied patients had maximum nocturnal GH peak below 10μg/L and four of them had mean nocturnal GH concentration below 2μg/L. Two had severe neurosecretory dysfunction of GH secretion (patient 6, 10). Their circulating IGF-I and IGFBP3 concentrations were markedly reduced compared to those for controls.

Correlation between circulating hormonal and ferritin concentrations for all the study children are presented in table 5. Serum ferritin concentration was correlated significantly (negatively) with mean nocturnal GH , IGF-I, IGFBP3 and insulin concentrations. Mean nocturnal GH concentration and the peak nocturnal GH level were correlated significantly with IGF-I and IGFBP3 supporting the view that GH is the major regulator of both IGF-I and IGFBP3 synthesis.

MRI studies revealed marked reduction of the pituitary volume in thalassemic children (305 +/- 125 mm3) versus age-matched normal controls (618 +/- 87 mm3) ($p<001$). Those patients with GH deficiency had variable abnormalities including: complete empty sella (n= 2) , marked diminution of the pituitary size (n=4), focal hypo/isointense lesions within anterior lobe pituitary gland at T1 and markedly hypointense at T2 (n=4) with variable sized focal hypo/isointense lesions within the midbrain at T1 and markedly hypointense at T2 (n=6) Consistent with *heamosedrin* deposition. None of the control children had abnormalities of the pituitary gland or its stalk.

DISCUSSION

The growth promoting activity of IGF-I is determined not only by the concentration of IGF-I but also by the amounts of various IGF binding proteins (IGFBPs).(21,22) Among these IGFBP3 is the major binding protein. (23) IGFBP3 has been observed to potentiate the effects of IGF-I in bone. (24-26) In addition, recent data clearly indicate that IGFBP3 prolongs the half life of circulating IGF-I levels and changes the clearance pattern of plasma IGF-I.(27) Current opinion favours GH as the major regulator of IGF-I and IGFBP3 in humans. In this study, the GH responses to provocation with clonidine and glucagon was impaired in short prepuberatal children with beta thalassaemia major compared to those for short normal children. Seven out of the 17 children with thalassaemia did not secrete GH after provocation (peak GH <7 ug/L) in both provocative tests. Five of the children had normal GH response to provocation , but abnormal nocturnal spontaneous GH secretion. MRI studies of the hypothalamic-pituitary area of 12 patients with thalassemia (7 with GH deficiency, 2 with abnormal spontaneous nocturnal GH release and 3 wit normal GH secretion) revealed complete empty sella (n= 2) , marked diminution of the pituitary size (n=4) , abnormal signal pattern of the gland (n=4) and thinning of the pituitary stalk (n=3) with its posterior displacement (n=2) in those patients with defective GH secretion (Fig 2) with relatively normal hypothalamic area, proving a good correlation between the structural alteration of the pituitary gland and dysfunction/deficiency of GH secretion in these patients. In support to our data previously published autopsy studies showed marked histopathological changes with significant siderosis of the pituitary gland and secondary atrophy of the somatotrophs. (6) The increased incidence of defective GH secretion in our thalassemic children, compared to other studies (9-12,28), could be explained on the basis of incomplete iron chelation in our patients with more siderosis of the pituitary gland.

All our patients with thalassemia had significantly lower concentrations of circulating IGF-I and IGFBP3. Impaired GH secretion can explain in part the significantly lower IGF-I and IGFBP3 synthesis with subsequent growth impairment in children with thalassaemia major. However, haemosiderosis of the liver in these patients with disturbed hepatic function might also decrease synthesis of IGF-I and IGFBP3. In our study serum ferritin concentration was significantly correlated with IGF-I concentration ($r = - 0.47$, $P< 0.01$), and IGFBP3 ($r=-0.43$, $p< 0.01$). This supports the view that siderosis of the liver can adversely affect the IGF-I/IGFBP3 synthesis. Deficiency of IGFBP3 in thalassemic patients might contribute to their growth impairment by decreasing the growth promoting effects of IGF-I.

We (29) and others (30) reported progressive impairment of insulin secretion in children with thalassaemia due to haemosiderosis of the pancreas. Other investigators stressed as well the importance of insulin resistance in these patients. (30,31). Insulin deficiency and/or resistance might be a contributing factor compromising the growth potential of these children through inhibition of IGF-I synthesis and/or secondary to increased production of inhibitory IGF-binding proteins. In this study, although the fasting serum insulin concentrations were within the normal range, they were correlated significantly with circulating IGF-I and IGFBP3 levels.

Delayed or arrest of puberty is common in patients with thalassaemia (4,5) due to disturbed gonadotrophins secretion (5,34) with consequent deficiency of sex-steroids. Sex steroids can influence growth through the modulation of GH secretion and IGF-I-induced cellular response (35-37) and consequently their deficiency impairs linear growth and attenuates the pubertal growth spurt in thalassaemic children.(38)

The finding of impaired linear growth in thalassaemic children, despite regular transfusion and desferroxamine therapy, who have normal GH secretion suggests the possibility of GH resistance. In a previous report our group studied IGF-I generation in a group of thalassemic patients (n=15) and found that they do not secrete adequate IGF-I after GH stimulation when compared to normal short children or those with growth hormone deficiency (GHD). After one year of GH therapy, despite giving the same dose/m2 to all the children, thalassemic children had significantly lower IGF-I concentrations versus those for the short normal control group and those with GHD.(39) These data are in concert with those of Werther GA et al (40) who reported lack of response of nonsuppressible insulin-like activity to short-term administration of human growth hormone in their thalassaemic patients. We treated 6 thalassaemic children with GH for a year. Their growth velocity increased significantly from 3.8 +/- 0.6 cm/yr to 7.2 +/- 0.8 cm/yr (doubling). However, the increment of the rate of growth was significantly slower compared to a control group with CSS. Collectively these findings suggest the presence of significant GH resistance in these patients which could attenuate the growth promoting effects of GH therapy. In concert with our view, Low LC et al (38) have shown that with higher (supraphysiological) (30units/m2/week divided on daily S.C. doses) doses of exogenous GH there has been a progressive increase of IGF-I production in their thalassaemic patients. Although it is known that higher GH doses during treatment possibly elicit a higher IGF-I and GV response, however this high dose might be necessary in thalassemic children to overcome the possible GH resistance. However, supraphysiological doses of GH might increase the risk of inducing diabetes and hypertension (29,30,39) in these high risk patients. Human IGF-I therapy , alone or in combination with GH and/ or IGFBP3 , appears to be an attractive alternative to overcome the GH resistance and avoid the high risk of developing diabetes in them. In human (41,42) and animal (26,27) experiments this combination of growth factors appear to be useful.

In summary; children with beta-thalassaemia and short stature have high incidence of abnormalities involving the GH/IGF-I/IGFBP3 axis with low circulating IGF-I and IGFBP3 concentrations. Decreased GH response to provocation (7/15) and abnormal properties of spontaneous nocturnal GH secretion (5/15) have been detected in this study and appear to be important etiological factors. In these patients defective GH secretion is associated with abnormal structure of the pituitary gland and its stalk. These changes are likely to be secondary to hemosiderosis of the pituitary gland as evidenced by the presence of iron deposits in the pituitary gland (with different degrees of pituitary atrophy), as well as in the mid-brain of these patients. However, children with thalassemia who have normal GH response to provocation and normal spontaneous (mean and integrated) GH secretion still have low circulating IGF-I concentrations suggesting partial resistance to GH. Early treatment of the defective GH/IGF-I/IGFBP3 axis in these children might markedly improve their linear growth, however, these patients usually require supraphysiological doses of growth hormone.

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Table 1: Auxologic data of patients and controls

	No	Sex	Age	B-age delay	HtSDS-1	HtSDS-2	U/L segment	GV	BMI
		M/F	yr	yr	(-)	(-)		cm/yr	kg/m2
lassemia	15	9/6	14.8 +/- 2.5	2.7 +/- 0.4	2.3+/- 0.65	2.45 +/- 0.55	.78+/- 0.05	4 +/- 1.3	17.8 +/-2.5
CSS	15	8/7	14.2 +/- 1.8	2.5 +/- 0.8	1.9+/- 0.3	2.2+/- 0.5	1+/- 0.07*	4.6 +/- 0.6	16.5+/-1.6

B-age= bone age, HtSDS= height standard deviation score, 1 and 2 = before and after 1 year,

GV= growth velocity , *p <0.05 among groups

Table 2: Biochemical and hormonal data (mean +/- SD)

	Ferrit	Hb	ALT	Bili	Alb	Ca	PO4	ALP	FT4	TSH	Insu	Cor
	ηg/ml	g/dl	U/L	μmo l/L	g/L	ηmo l/L	ηmo l/L	U/L	ρmo l/ml	μU/ ml	mIU /ml	ηmo l/L
Thal	1,455*	7.8*	88*	24*	41	1.9	1.4	188	14.7	3.2	10.2	311
n=15	411	1.3	38	6	5.4	0.06	0.03	54	3.4	0.8	5.7	78
CSS	105	13.3	18.7	5.8	39	2.2	1.5	165	17.5	1.5	12.2	382
n=15	44	1.1	11.5	4.5	6.2	0.03	0.08	49	2.9	0.5	4.5	85

*p below 0.05, ferrit= ferritin, alb = albumin, Insu = fasting insulin , Cor= 8:00 A.M. cortisol.

Table 3: GROWTH FACTORS CONCENTRATIONS IN PATIENTS AND CONTROLS

(mean +/- SD)

	Thalassemia	CSS
	n=15	n=15
IGF-I (ng/ml)	68.5 +/- 19	153 +/- 42*
IGFBP3(mg/L)	1.22 +/- 0.27	2.16 +/- 0.37*
P-GH to clonidine (ug/L)	8.8 +/- 2.3	17.6 +/- 2.7*
P-GH to glucagon (ug/L)	8.2 +/- 3.1	15.7 +/- 3.7*

• $p < 0.05$. IGF-D= the response of IGF-I above basal levels after GH injection, P-GH= peak GH response

Table 4: Anthropometric data of thalassemic patients with normal GH response to provocation.

Name	Sex	Age	BMt	Ht	Span	U/L	HtSDS	MAC	HC	Breast	Testes
		yr		cm	cm		(-)	cm	cm		diam cm
S.G	M	13.5	16.5	135	136.7	0.85	2.5	20	53	-	1.5
Mo.S	M	14	14.9	142	141	0.87	2.25	17.2	55	-	1.2
N.R	F	17	18.6	143	146.3	0.85	3.16	23.5	56	I	-
F.R	F	13	18.3	134	137.5	0.74	2.6	21.4	53.5	I	-
M.S	F	13.5	14.3	140	142	0.77	2.7	18.2	52	I	-
S.M	F	25	18.4	152	155.2	0.71	1.9	21.4	56.2	III	-
D.H.	F	18	24	150	152.4	0.73	2.03	23	55.4	III	-
A.A.	F	14	19.7	133	135	0.8	4.1	20.2	53	I	-
N.A	M	13	18.7	136	142	0.81	1.9	20	54	-	1.4
M.G.	M	16	18.5	153	155	0.82	2.75	21.4	56	-	1.4
Mean	4M/6F	15.7	18.2	141.8	144.3	0.79	2.58	20.6	54.4		
SD		4.36	2.7	7.2	7.17	0.05	0.8	1.85	1.42		

Table 5: Pulse properties in thalassemic patients(n=8) and controls (n=3) {mean +/- SD}

	IGF-I	IGFBP3	Integ-N-GH	Mean-N-GH	Pulse Amplit	Max.N.Peak	Pulses
	ng/ml	mg/L	ug/L	ug/L	ug/L	ug/L	/12 hr
Thalassemics	58.9	1.12	2.53	2.94	9.2	9.3	2.8
	18	0.22	1.6	1.77	2.2	3	1.1
CSS	168	2.1	4.9	5.61	17.2	25.1	4.66
	45	0.44	0.29	0.52	2	1	0.94
Gh pulse = or >5 ug/L							

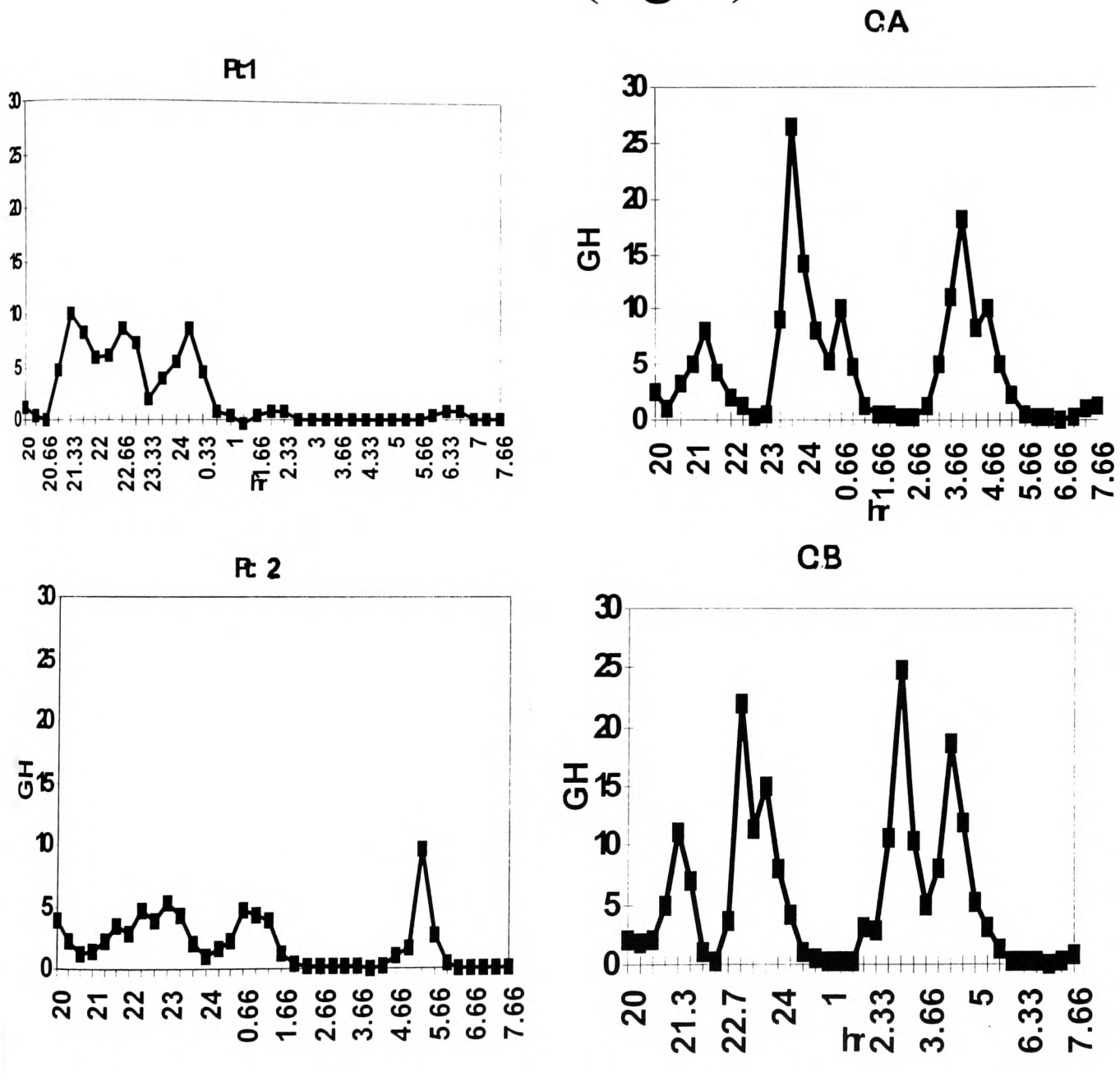
Integ-N-GH= ntegrated nocturnal GH, pulse-Ampli= mean pulse amplitude, Max-N-peak= maximum nocturnal pe

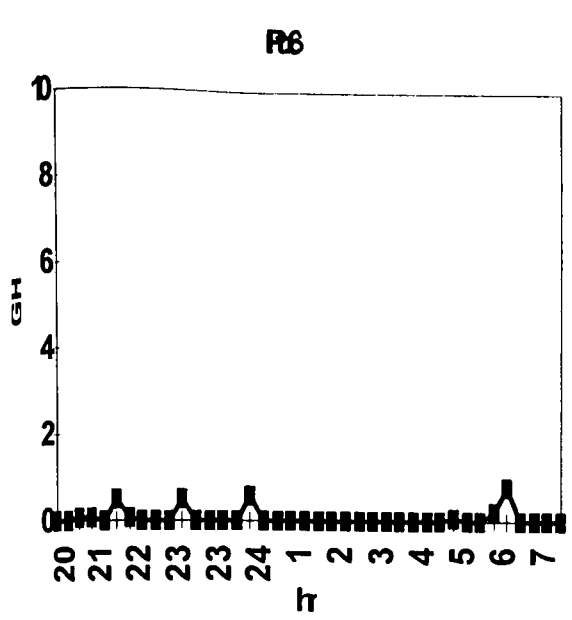
Table 6. Correlation between serum ferritin and hormone concentrations.

	Max. noct. peak	Mean noct. GH ug/L	IGF-I ng/ml	IGFBP3 mg/ml	Insulin ulU/ml
Ferritin ng/ml	-0.36*	-0.33*	-0.47**	-0.43*	-0.39*
IGF-I ng/ml	0.53**	0.578**	1	0.685**	0.52**
IGFBP3 mg/ml	0.47**	0.621**	0.657**	1	0.37*

*p <0.05, **p <0.01

Figure 1: Nocturnal GH secretion in thalassaemic patients (left) and controls (right)

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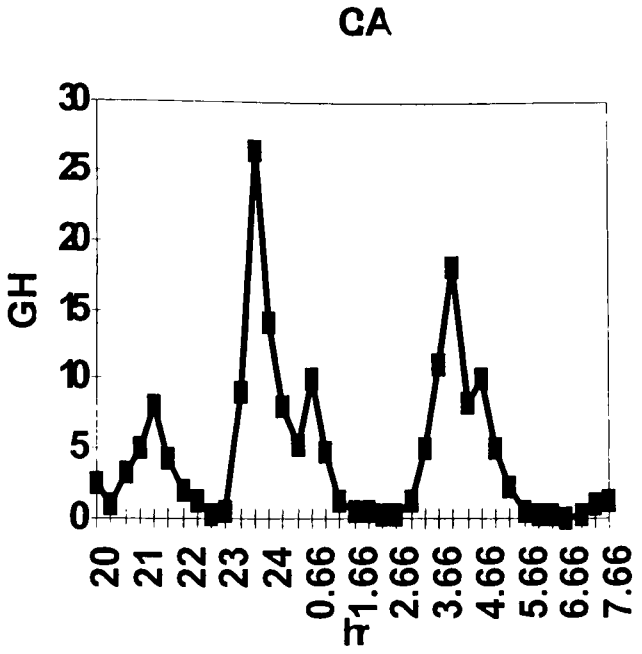
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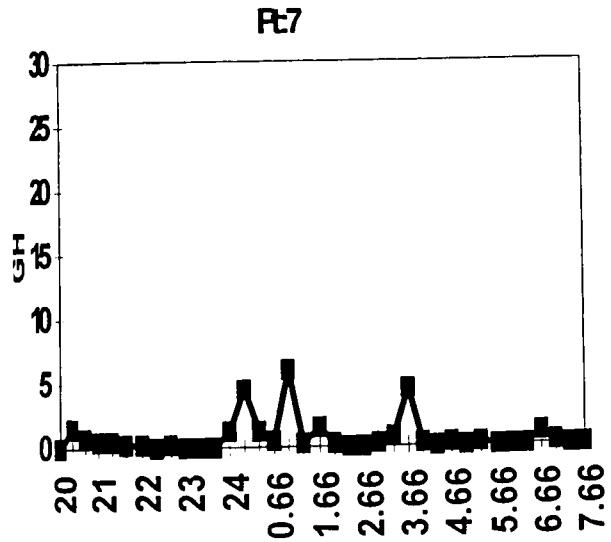
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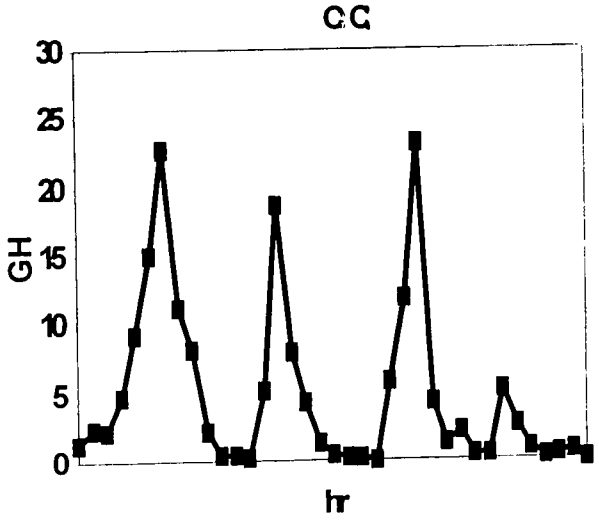
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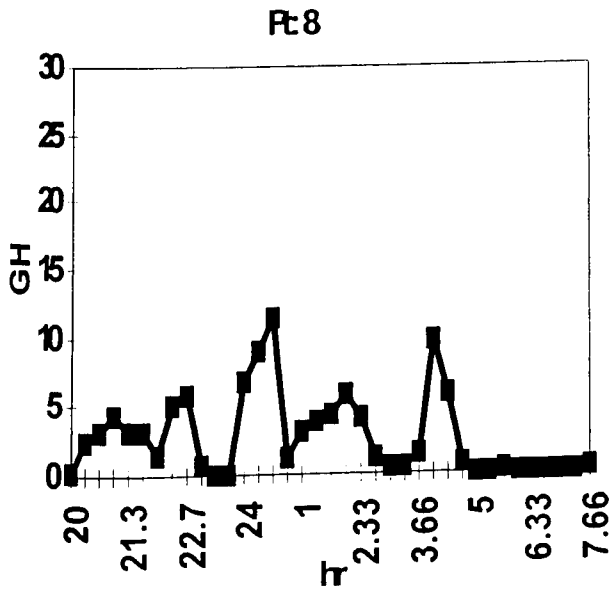
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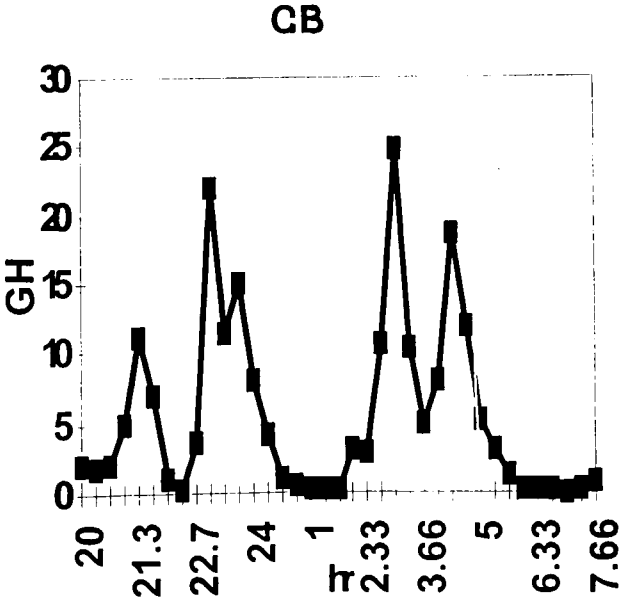
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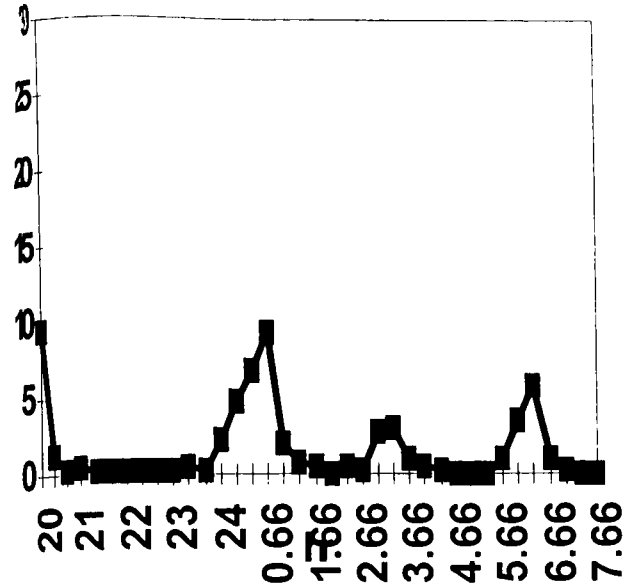

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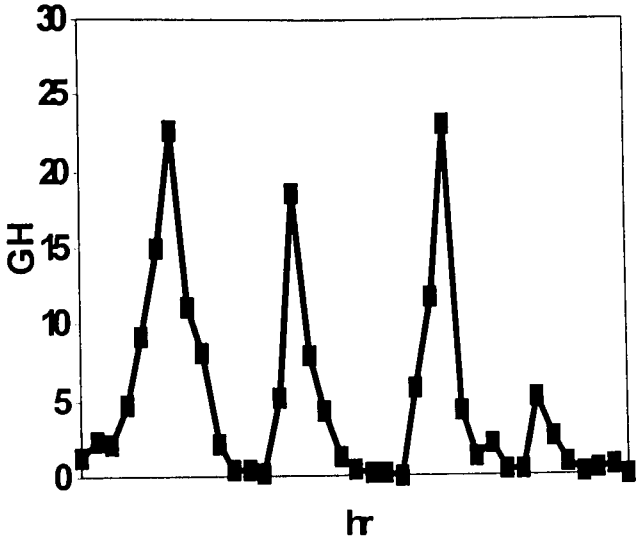

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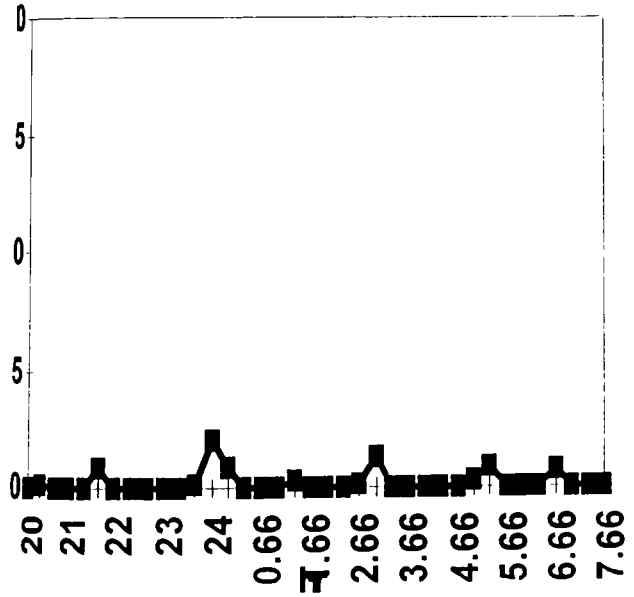

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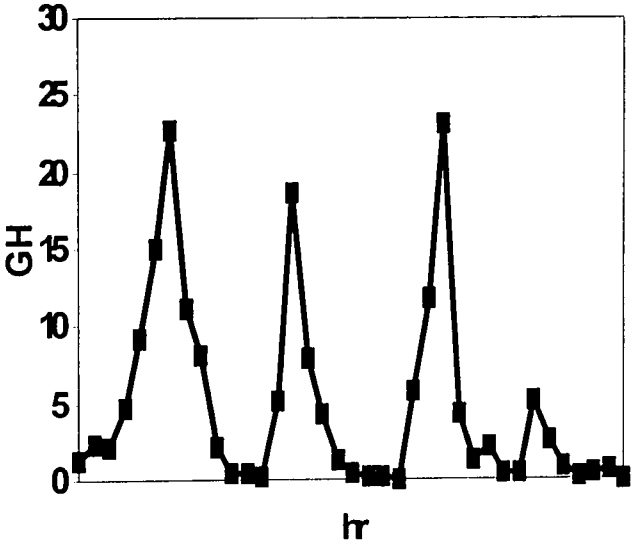
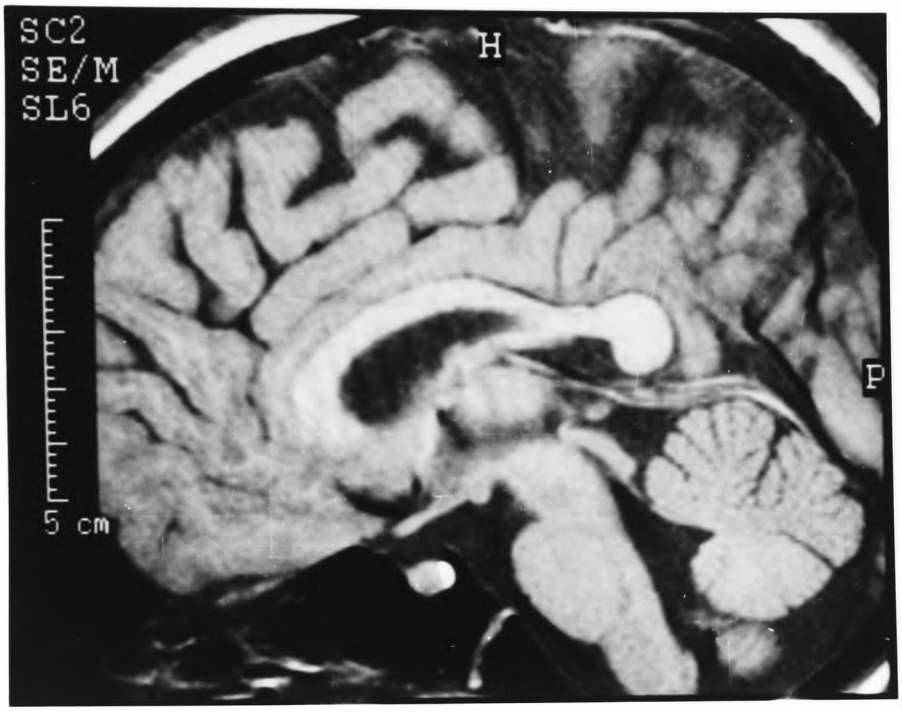


FIGURE 2



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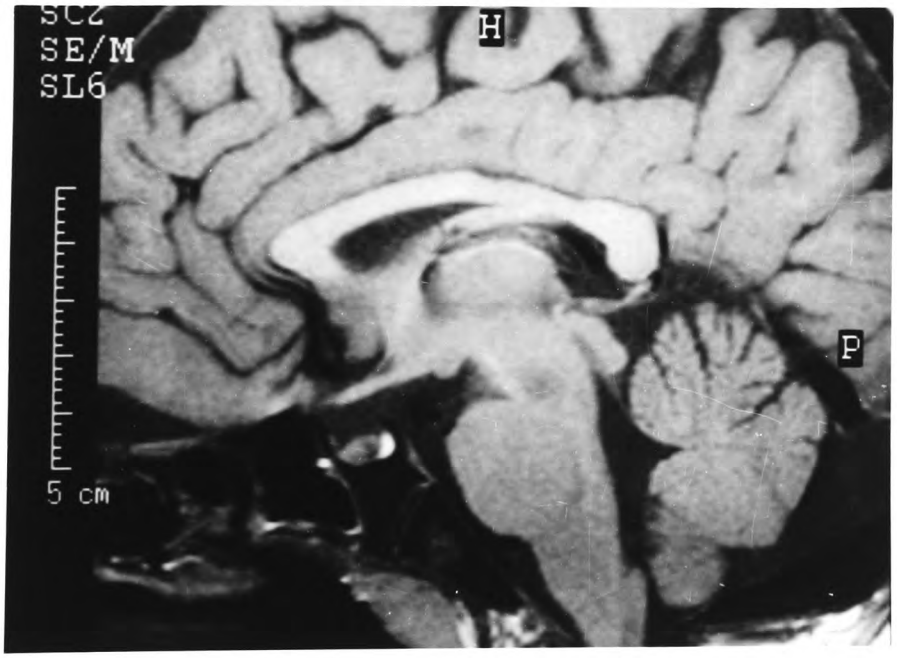

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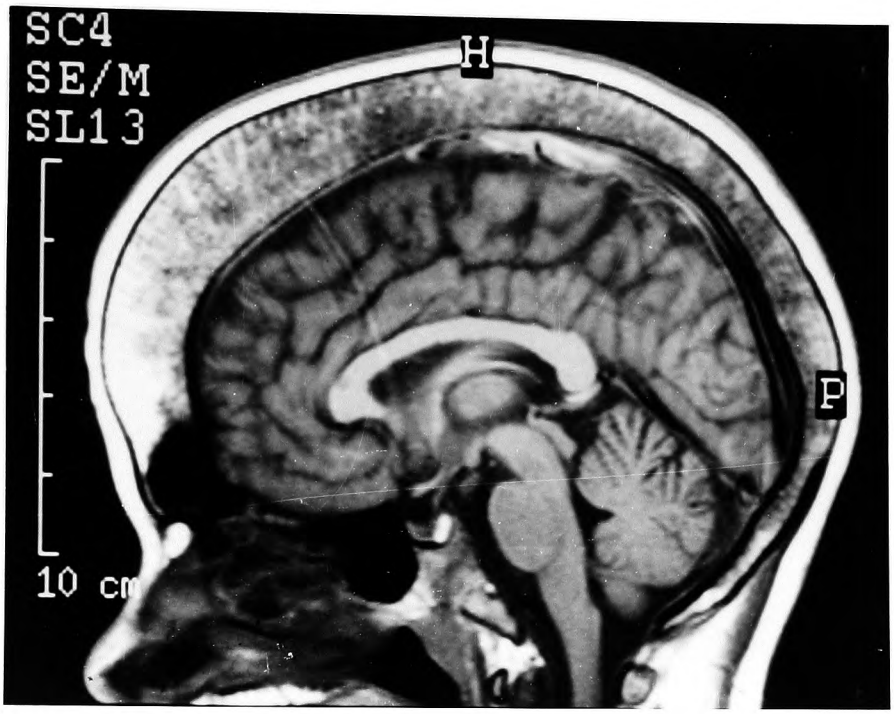

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FIGURE LEGENDS

Figure 1:

Nocturnal 12-hour profile of plasma GH (ug/L) in some patients with thalassemia major who had normal GH response to provocation and defective overnight GH secretion compared to controls (1-3)

Patient 1: Age= 13.5 years, HtSDS= -2.5, peak GH response =8.9 ug/L

Patient 6: Age = 25 years, HtSDS = -1.9, peak GH response = 8.1 ug/L

Patient 7: Age= 18 years, HtSDS = -2, peak GH response = 9.4 ug/L

Patient 8: Age= 14.5 years, HtSDS = -4.1, peak GH response = 7.8 ug/L

Patient 9: Age= 13 years, HtSDS = -1.9, peak GH response = 9.9 ug/L

Patient 10: Age= 16 years, HtSDS = -2.75, peak GH response = 9.1 ug/L

Control 1: Age= 14 years, HtSDS = -1.8, peak GH response = 16.2 ug/L

Control 2: Age= 15 years, HtSDS= -1.8, peak GH response = 12.7 ug/L

Control 3: Age= 16 years, HtSDS= -2.2, peak GH response= 14.8 ug/L

Figure 2:

MRI images in thalassaemic patients

SE 600/20 T1 weighted image sagittal scan at the level of the mid-pituitary gland demonestrating:

2-A: normal volume anterior and posterior pituitary lobes, with ill-defined iso/hypointense focal lesion identified within the lower part of mid-brain and partial atrophic changes of the cerebral cortex .

2-B: slightly diminished volume of the anterior pituitary lobe, normal posterior pituitary, focal hypo/ isointense area identified within the anterior pituitary substance and the lower part of the mid brain with increased CSF within the dorsal part of the sella tursica and suprasellar cistern. Partial atrophic changes of the cerebral cortex.

2-C: markedly diminished volume of the anterior pituitary lobe and normal posterior pituitary with relatively large focal hypointense area within the remenant of the anterior pituitary lobe and small hypo/isointense focal area within the lower part of the mid-brain and increased CSF at the dorsal aspect

of the sella turcica and suprasellar cistern (partial empty sella). The calvarium is markedly thickened due to increased diploic space.

2-D: Total loss of the anterior pituitary lobe and mild posterior hooking of the lowermost part of the pituitary stalk. Normal volume of the posterior pituitary. There is an iso/hypointense focal lesion at the lower part of the mid-brain. The anterior part of the sella tursica is filled with CSF (empty sella).

2-E: Total loss of the anterior pituitary lobe with normal posterior pituitary with empty sella and focal iso/hypointense area in the lower part of the mid-brain. Partial atrophic changes of the cerebral cortex are observed.

2-F: Sagittal SE 2000/80 T2 weighted image at the level of the mid-pituitary demonstrating marked hypointensity of the anterior pituitary, large focal area of marked hypointensity identified within the midbrain, partial atrophic changes of the cerebral and cerebellar cortices.

Bone Mineral Density in Prepubertal Children With β-Thalassemia: Correlation With Growth and Hormonal Data

Ashraf T. Soliman, Nagwa El Banna, Mohammed Abdel Fattah, Mahmoud M. ElZalabani, and B.M. Ansari

Patients with β-thalassemia major (β-thalassemia) frequently have bone disorders of multifactorial etiology. We attempted to analyze the relationship between the bone mineral density ([BMD] measured by dual-photon absorptiometry) and auxanologic parameters, degree of siderosis, function of the growth hormone (GH)/insulin-like growth factor-I (IGF-I)/IGF-binding protein-3 (IGFBP3) axis, calcium-phosphate balance, parathyroid hormone (PTH), and cytokines (interleukin-1β [IL-1] and tumor necrosis factor-alpha [TNF-α]) in 30 prepubertal children with β-thalassemia major and 15 age-matched children with constitutional short stature (CSS), who have normal glucose tolerance and thyroid function. Children with β-thalassemia had a significantly decreased BMD and mean BMD% for age and sex (0.75 ± 0.24 g/cm² and 71% ± 10%, respectively) versus children with CSS (1.06 ± 0.3 g/cm² and 92% ± 7%, respectively). Thalassemic patients had significantly lower circulating concentrations of IGF-I and IGFBP3 (49 ± 21 ng/mL and 1.2 ± 0.25 mg/L, respectively) compared with control children (153 ± 42 ng/mL and 2.1 ± 0.37 mg/L, respectively). The GH response to provocation by clonidine and glucagon was defective (peak GH < 7 μg/L) in 12 of the 30 thalassemic children. Serum concentrations of IL-1β and TNF-α did not differ among the two study groups. Hypocalcemia was detected in five of the 30 thalassemic patients: hypoparathyroidism was diagnosed in two of the five and rickets in the other three. BMD was highly correlated with the circulating concentrations of IGF-I and IGFBP3, as well as with the auxanologic parameters (age, weight, height, height standard deviation score [HSDS], and body mass index [BMI]). It is suggested that increasing the circulating IGF-I concentration through aggressive nutritional therapy and/or GH/IGF-I therapy with supplementation with vitamin D and/or calcium might improve bone growth and mineralization and prevent the development of osteoporosis and consequent fractures in these patients. Such therapy requires blinded controlled trials.

OSTEOPOROSIS is a disease characterized by loss of bone mass and microarchitectural deterioration, resulting in a reduced mechanical competence and consequent increased risk of fractures.[1] β-Thalassemia major is associated with significant bone disease.[2] These changes include bone marrow expansion of the medullary cavity, cortical thinning, trabecular coarsening with various striations or the appearance of cystic spaces, and coarsening of the bone pattern with a drop out of all but the mechanically most necessary trabeculae.[3]

With advances in transfusion management beginning in the 1960s, there has been marked improvement in terms of skeletal development and normal cosmetic facial and long bone appearance.[4] However, even well-transfused patients remain radiographically osteopenic. Prior to institution of aggressive transfusion regimens, fractures occurred primarily in the long bones and were associated with trauma.[5] After the introduction of aggressive transfusion, the pattern changed, with less involvement of long bone and an increased in vertebral compression fractures, especially in older patients.[6]

Although the etiology of the bone disease is still debatable, many factors can adversely affect bone accretion in thalassemic children. These include (1) chronic hypoxemia and medullary expansion, (2) defective growth affecting both height and weight, (3) abnormal calcium-phosphate homeostasis, (4) delayed or lack of pubertal development and decreased sex steroid secretion, (5) compromised nutritional status and increased energy expenditure, (6) abnormal growth hormone (GH)/insulin-like growth factor-I (IGF-I)/IGF-binding protein-3 (IGFBP3) axis, and/or (7) development of diabetes mellitus.[7-10]

The aim of this study was to investigate some factors affecting bone mineral metabolism in 30 children with β-thalassemia and to attempt to find a relationship, if any, between the degree of siderosis, calcium-phosphate balance, the GH/IGF-I/IGFBP3 axis, parathyroid hormone (PTH) secretion, and auxanologic data, on one hand, and bone mineral density (BMD) on the other.

SUBJECTS AND METHODS

Thirty-three prepubertal patients with β-thalassemia major randomly selected from those attending the outpatient Pediatric Hematology Clinic of Alexandria University Children's Hospital, Alexandria, Egypt, were the subjects of this study. All children underwent regular blood transfusions to keep the hemoglobin (Hb) concentration above 10 g/dL. All were taking folic acid supplements and iron chelation with daily intramuscular desferoxamine. Fifteen age-matched normal short children (constitutional short stature [CSS], height standard deviation score [HtSDS] ≤ −2, annual growth velocity [GV] ≤ 5 cm/yr, normal GH response to provocation, and delayed bone age) served as controls. None of the children had a history of intrauterine growth retardation, other systemic or endocrine disease or dysmorphic trait, or central nervous system irradiation. All had normal tolerance to an oral glucose load (1.75 g/kg dextrose). Three patients who had abnormal glucose tolerance were excluded from the study.

Informed consent for the testing procedures was obtained from the parents and, when appropriate, from the children before entering the study. The study protocol was approved by the ethics committee of Alexandria University.

All children were examined with a special emphasis on nutritional data. The auxanologic data included weight, height, and midarm circumference. Harpenden calipers and anthropometric measurements were used. The data recorded were the average of three sequential measurements determined by the same observer (A.T.S.). The HtSDS and body mass index (BMI) were calculated and recorded. The linear GV in centimeters per year was calculated for the past year. Normal

From the Department of Pediatrics, University of Alexandria Children's Hospital, Alexandria, Egypt; and Department of Pediatrics, East Glamorgan General Hospital, Church Village and School of Applied Sciences, University of Glamorgan Pontypridd, Wales, UK.

Submitted May 14, 1997; accepted November 14, 1997.

Address reprint requests to Ashraf T. Soliman, MD, Associate Professor of Pediatrics, University of Alexandria Children's Hospital, 3 Abdel Sattar Mansour St, Loran, Alexandria, Egypt.

0026-0495/98/4705-0009$03.00/0

population data were from Tanner et al.[11] The bone age was determined according to the Greulich and Pyle atlas.[12]

On the day of admission, venous blood samples were obtained for determination of the complete blood cell count, and serum concentrations of albumin, bilirubin, and alanine aminotransferase (ALT). Following an overnight fast (8 hours) venous blood samples were drawn through a polyethylene catheter inserted in a forearm vein between 8 and 9 AM. The serum was separated from the formed elements by centrifugation and kept frozen at −20°C until analyzed for GH, IGF-I, IGFBP3, free thyroxine (FT_4), thyrotropin (TSH), cortisol, PTH (intact molecule), calcium (Ca), phosphorus (PO4), alkaline phosphatase (ALP), ferritin, interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF = α) concentrations. After obtaining the basal samples, an oral dose of clonidine 0.15 mg/m^2 and intravenous dose of corticotropin (ACTH) (Synacthen; Ciba-Geigy, Basel, Switzerland) 1 μg/m^2 were given, and blood samples were collected every 30 minutes for 2 hours for measurement of GH levels and after 60 minutes for cortisol levels. On the next morning, a standard glucagon test for GH release was performed.

Human GH and IGF-I concentrations were measured by radioimmunometric assay using reagents purchased from Nichols Institute (San Juan Capistrano, CA). Mean intraassay coefficients of variation (CVs) were 5.8% and 7.6%, respectively, and interassay CVs were 7.8% and 8.5%, respectively, in the range of GH and IGF-I values detected. The IGFBP3 level was measured by radioimmunoassay in Serono Biochemical Laboratories (SCL) & Bioscience Services (London, UK) using reagents supplied by Mediagnost (Rome, Italy). The assay sensitivity is 0.06 μg/mL with intraassay and interassay CVs of 5.2% and 8.6%, respectively. The level of intact PTH was measured in the serum using an immunochemiluminometric method. Intraassay and interassay CVs were 3.6% and 6.2%, respectively. IL-1β and TNF-α levels were measured using an enzyme-linked immunosorbent assay technique (Biokine; T-Cell Diagnostics, Cambridge, MA; intraassay CVs, 8.4% and 6%, respectively; interassay CVs, 8.8% and 7%, respectively).

BMD of the lumbar spine (second, third, and fourth lumbar vertebrae) was measured by dual-photon absorptiometry using a Norland 2600 bone densitometer (Cambridge, UK). All children were scanned in the supine position. BMD data were expressed as grams per centimeter squared and were compared with BMD values of normal children of the same age.[13]

Statistical analyses were performed using the unpaired *t* test to compare mean analyte concentrations among the two study groups when the data were normally distributed, and the Wilcoxon test when they were not. Statistical significance was accepted at P less than .05. Multiple regression analysis was performed using BMD as the dependent variable and all of the other auxanologic and biochemical data as independent variables. Data are presented as the mean ± SEM.

RESULTS

Anthropometric and bone age data are presented in Table 1. The chronological age, HtSDS, GV, BMI, and bone age did not differ significantly between the two study groups. Biochemical, hormonal, and BMD data are shown in Tables 2 and 3. The circulating concentrations of albumin, creatinine, ALP, and PO4 did not differ significantly among the two groups. Hypocalcemia (Ca ≤ 1.4 nmol/L, 5.7 mg/dL) was detected in five patients. Three of them (aged 11, 12.5, and 14 years) had other biochemical evidence of hypoparathyroidism (high PO4, normal ALP, and low PTH concentrations). The other two patients (aged 9 and 11 years) had biochemical evidence of rickets (low PO4, high ALP and PTH, and low 25-hydroxyvitamin D_3 concentrations). Circulating concentrations of IGF-I and IGFBP3 were significantly lower in thalassemic children compared with controls. Their peak GH responses to provocation with clonidine and glucagon were significantly less than those for the controls. Twelve of the 30 children with β-thalassemia did not mount a GH response to provocation above 7 μg/L. Although basal levels of cortisol did not differ between the two groups, the cortisol response to a low-dose ACTH test was significantly lower in thalassemic children versus controls. Two children had mild chemical hypothyroidism (FT_4, 9.5 and 8.7 pmol/L; TSH, 8.6 and 10.3 μIU/mL). Both were treated with L-thyroxine for 1 month before testing their GH response to provocation.

Table 1. Anthropometric Data of the Patients and Controls

Group	HtSDS	GV (cm/yr)	Age (yr)	BMI (kg/m^2)
β-Thalassemia (n = 30)	1.95 ± 0.11	4.1 ± 0.24	8.8 ± 0.27	13.9 ± 0.24
CSS (n = 15)	2.6 ± 0.13	4.6 ± 0.03	8.2 ± 0.26	14.9 ± 0.013

*P < .05, β-thalassemia *v* CSS.

Dual-photon absorptiometry showed that children with β-thalassemia had a significant reduction of BMD (30% less) compared with the mean BMD for age-and sex-matched normal children, corresponding to a BMD of −1.5 to −2 SD. Thalassemic children had significantly lower BMD versus age-matched children with CSS. Correlations between BMD and different parameters are shown in Table 4 and Fig 1. BMD was correlated significantly (P < .01) with age, height, weight, and BMI, as well as with the circulating concentrations of IGF-I and IGFBP3. No significant correlations were found between BMD, on one hand, and PTH, PO4, Ca, or ALP concentrations on the other. IL-1β and TNF-α concentrations did not differ significantly between the thalassemic group (25.9 ± 11.4 and 399 ± 113 pg/mL, respectively) and controls (21.1 ± 6.4 and 383 ± 122 pg/mL).

DISCUSSION

From infancy through late adolescence, bone-forming activity exceeds bone resorption, resulting in a steady accumulation of bone mass. On average, most of the skeletal mass is accumulated by the age of 18 years.[14-18] Since the bone mass is one of the main determinants of fractures, a high bone mass at skeletal maturity (peak bone mass) is considered the best protection against age-related bone loss.[19] Small differences in bone mass at skeletal maturity of 5% to 10% could contribute to substantial differences in the incidence of osteoporotic fractures.[20]

Bone modeling and skeletal consolidation result from a complex sequence of hormonal changes in interaction with nutritional factors, where the concerted actions of GH, IGF-I, and sex hormones and their receptors, besides other factors, are responsible for the timing and attainment of skeletal consolidation. At puberty, circulating IGF-I concentrations correlate with sexual development. Specifically, the surge in sex steroids, in turn, increases the secretion of GH, which stimulates the production of IGF-I[21,22] and increases bone mass.[23] In addition, a large number of other factors interact at the level of the osteoblast, osteoclast, and other cells to regulate the balance between net resorption and formation. These include PTH, vitamin D, and cytokines.[23]

Table 2. Biochemical Data of the Patients and Controls (mean ± SEM)

Group	Albumin (g/dL)	ALT (IU/L)	Bilirubin (μmol/L)	Ca^{2+} (mg/dL)	PO4 (mg/dL)	ALP (IU/L)	Ferritin (ng/mL)	Hb (g/dL)
β-Thalassemia (n = 30)	3.8 ± .09	86 ± 5*	49 ± 2.7*	8.2 ± .15	5 ± .2	178 ± 10	880 ± 46*	8.2 ± .27*
CSS (n = 15)	3.9 ± .1	21 ± 3	15 ± 1.3	9.1 ± 0.3	4.4 ± 013	161 ± 7	89 ± 10	12.4 ± 0.3

*$P < .05$, β-thalassemia *v* CSS.

The GH/IGF-I/IGFBP3 Axis and Factors Affecting It

In this study, the hormonal profile of children with β-thalassemia showed a significant deficiency of circulating IGF-I and IGFBP3 (both are GH-dependent peptides). The significant correlation between the IGF-I levels and HtSDS and BMD supports a major role played by IGF-I in stimulating linear growth and bone mineralization.

Forty percent of these prepubertal thalassemic children had defective GH secretion after provocation by clonidine and glucagon. In concert with our findings, Danesi et al,[24] Saglamer et al,[25] and Pintor et al[26] reported a low GH response to provocation by insulin hypoglycemia, arginine, L-dopa, and GH-releasing hormone in many of their patients, denoting impairment of somatotroph function. This can explain the low IGF-I synthesis in some patients. However, the thalassemic children with normal GH secretion had low circulating IGF-I concentrations (50 ± 19 ng/mL) comparable to those seen with defective GH release (46 ± 24 ng/mL), suggesting that other factors contribute to low IGF-I synthesis in these children. Leger et al[27] found that decreased IGF-I secretion occurs before an alteration in GH secretion in response to GH-releasing hormone, arginine, or insulin. Other investigators reported the neurosecretory dysfunction of GH secretion to be responsible for decreased IGF-I synthesis in some patients with a normal GH response to provocation.[28,29] Some investigators indicated that decreased GH secretion may be due to an age-related deterioration of the hypothalamic-pituitary function secondary to progressive siderosis.[27,30] In support, Perignon et al[31] reported that their patients with β-thalassemia had a low IGF-I concentration that did not increase at puberty. Although the idea of a defect at the hepatic GH receptor or postreceptor level was suggested to explain the low IGF-I production,[26] Postel-Vinay et al[32] found no evidence for a defect in GH binding to liver membranes in thalassemic patients.

Table 3. Hormonal Data of the Patients and Controls

Parameter	CSS (n = 15)	β-Thalassemia (n = 30)
FT_4 (pmol/L)	18.4 ± 0.155	15.2 ± 0.347*
TSH (μIU/mL)	1.6 ± 0.038	2.5 ± 0.347
GH-P-Clon (μg/L)	19.6 ± 0.697	6.9 ± 0.49†
GH-P-Glu (μg/L)	16.1 ± 0.82	7.4 ± 0.4†
IGF-I (ng/mL)	153 ± 10.85	49 ± 3.8†
IGFBP3 (mg/L)	2.1 ± 0.09	1.2 ± 0.045†
Cortisol-b (nmol/L)	466 ± 18.8	318 ± 11.5*
Cortisol-a (nmol/L)	788 ± 25.6	455 ± 17.4†
PTH (pmol/L)	7.8 ± 1.16	11.1 ± 2.88
BMD (g/cm²)	1.06 ± 0.08	0.75 ± 0.044*‡
BMD (%)	92 ± 0.2	71 ± 1.8*‡

Abbreviations: GH-P-Clon, GH peak after clonidine; Glu, glucagon; cortisol-b, before ACTH; a, after ACTH.

*$P < .05$, †$P < .01$, β-thalassemia *v* CSS.

‡$P < .05$, β-thalassemia *v* normal children.[13]

It is well recognized that the nutritional status has an important influence on the GH/IGF-I/IGFBP3 axis.[33] Fasting results in increased GH secretion and decreased IGF-I levels,[34] and proper nutrition increases IGF-I levels in malnourished children.[35] Our children with β-thalassemia had a BMI and MAC at or below the 10th centile for age and sex, suggesting a mild degree of undernutrition. Decreased food intake,[36] pancreatic exocrine dysfunction, hepatic cirrhosis,[37,38] and/or hypermetabolism secondary to bone marrow hyperactivity and increased cardiac work might compromise nutrition and growth in these children. In this study, circulating IGF-I concentrations were correlated significantly with ALT levels ($r = -.465$, $P < .01$), and 50% of the children were hepatitis B surface antigen carriers and had significantly elevated ALT concentrations. Clinically, 25 of 30 patients had cirrhotic livers. In one study,[36] nutritional intervention resulted in an improvement of weight for height and increased IGF-I concentration. In malnutrition[35] and hypercatabolic states,[39] low IGF-I production is associated with high basal and stimulated GH levels, denoting a normal sensitivity of the hypothalamic-pituitary axis to the low IGF-I level (normal feedback). In β-thalassemia, the low-normal GH levels despite low circulating levels of IGF-I prove a defective-feedback effect of decreased IGF-I on the pituitary (either due to lack of sensitivity or defective somatotroph function). We and others reported partial resistance to GH in thalassemic children as evidenced by low IGF-I generation in response to exogenous administration of GH and slow linear growth on GH therapy.[40-42]

During a normal pubertal growth spurt, sex steroids increase GH secretion with a subsequent increase of IGF-I levels. Sex steroids and GH each contribute approximately 50% of the height gain. Children with GH insufficiency not treated with exogenous GH attain only 50% to 66% of the expected growth spurt.[43-47] Reduction of the sex steroid concentration during gonadotropic-releasing hormone therapy decreases GH secretion and serum IGF-I concentrations.[48,49] Patients with β-thalassemia have a high incidence of failure of puberty (51% of boys and 47% of girls) and secondary amenorrhea (23%).[50] Defective gonadotropin secretion with subsequent sex steroid deficiency have been detected in these patients with low IGF-I levels.[51-53] Even those who enter puberty do not have the

Table 4. Correlation Between BMD and Auxanologic and Biochemical Data (*r*)

	IGFBP3	IGF-I	BMI	HtSDS	Weight	Height	Age	Ferritin
BMD	.693†	.74*	.49†	.52†	.59†	.54†	.79†	−.27*
IGF-I	.72†	1*	.41*	.44†	.39*	.47†	.68†	−.45†

*$P < .05$.

†$P < .01$.

enhanced GH secretion and increased IGF-I synthesis pattern accompanying normal puberty,[30,31] denoting a major effect of sex steroid deficiency on the GH/IGF-I axis in peripubertal and pubertal children with β-thalassemia. Unlike children with a constitutional delay of puberty, who secrete normal GH in response to provocation after priming with sex steroid,[43] in our prepubertal thalassemic patients older than 12 years (n = 8), the peak GH response to provocation did not improve after (7.5 ± 2.1 μg/L) versus before priming with estrogen (6.5 ± 1.5 μg/L).

The reported prevalence of diabetes mellitus in treated β-thalassemia is about 16%, and the incidence of impaired glucose tolerance is approximately 60%. Islet cell destruction secondary to iron overload and/or exhaustion of B cells due to chronic insulin resistance and liver derangement are possible pathogenic factors.[54-58] Defective insulin secretion and an insulin-resistant state can impair hepatic IGF-I production.[59,60] Moreover, insulin plays an important part in determining the bioavailability of IGF-I through its action on IGFBP1. Therefore, defective insulin secretion or insulin resistance in thalassemic children can increase hepatic production of IGFBP1, leading to decreased bioavailability of IGF-I.[61,62] However, this factor can be excluded in our patients with normal glucose tolerance.

In summary, the markedly decreased hepatic production of IGF-I in our thalassemic prepubertal patients with normal

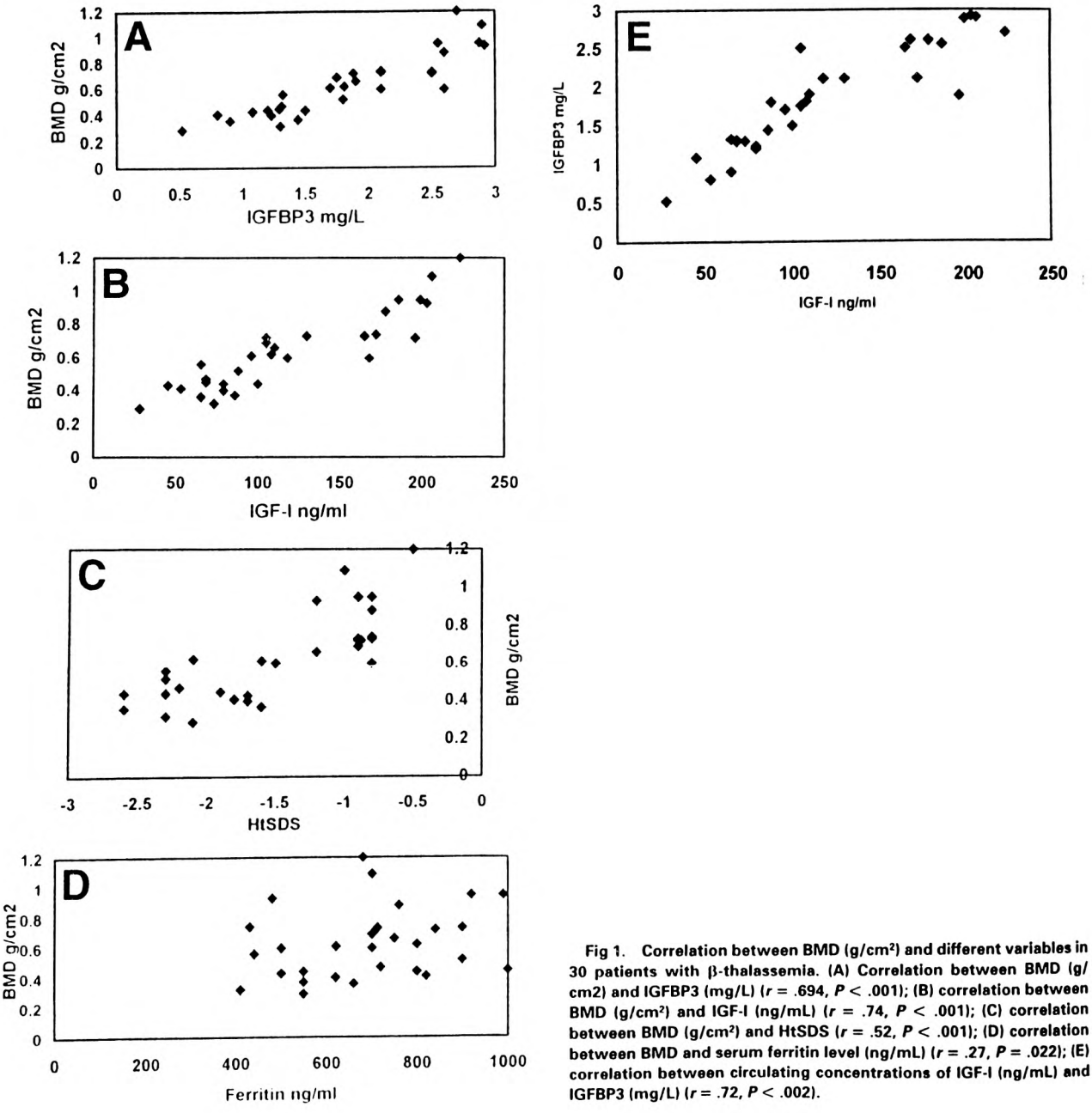

Fig 1. Correlation between BMD (g/cm²) and different variables in 30 patients with β-thalassemia. (A) Correlation between BMD (g/cm2) and IGFBP3 (mg/L) ($r = .694$, $P < .001$); (B) correlation between BMD (g/cm²) and IGF-I (ng/mL) ($r = .74$, $P < .001$); (C) correlation between BMD (g/cm²) and HtSDS ($r = .52$, $P < .001$); (D) correlation between BMD and serum ferritin level (ng/mL) ($r = .27$, $P = .022$); (E) correlation between circulating concentrations of IGF-I (ng/mL) and IGFBP3 (mg/L) ($r = .72$, $P < .002$).

glucose homeostasis can be attributed to defective GH secretion, hepatic cirrhosis (secondary to siderosis and/or chronic viral hepatitis), and/or GH resistance. The delay or lack of pubertal development and the occurrence of type I diabetes with advancing age might further impair the secretion and/or bioavailability of IGF-I.

IGF-I is a potent stimulator of linear growth and a major determinant of bone mineralization. Exogenous administration of IGF-I has been shown to increase growth and bone formation in humans and animals.[63-66] This effect is potentiated when IGF-I is combined with IGFBP3.[67,68] In our children with β-thalassemia, the decreased production of IGF-I and IGFBP3 and the significant correlation between these growth factors and BMD and linear growth (weight, height, and HtSDS) parameters suggested a major role for them in the pathogenesis of osteoporosis. GH or IGF-I therapy may improve linear growth and bone mineralization in these children, as seen in patients with GH deficiency/resistance and other diseases with osteopenia.[69-71] This hypothesis needs to be tested by a double-blind therapeutic trial of GH or IGF-I in these patients.

Calcium-Phosphate Balance and PTH

In this study, hypocalcemia occurred in five of 30 children with β-thalassemia. All five children had markedly decreased BMD. Analyses of the other biochemical parameters differentiated two possible disease entities. Two of the five patients had evidence of hypoparathyroidism (low Ca, high PO4, normal ALP, and low PTH). This can be explained by siderosis of the parathyroid gland.[72-75] In support of this view, Gertner et al[76] found a low PTH reserve to induced hypocalcemia in thalassemic patients. The other three patients had biochemical evidence of rickets (low PO4, low Ca, high ALP, high PTH, and low 25-hydroxyvitamin D_3). In concert with this finding, De Vernejoul et al[77] reported osteomalacia in their thalassemic patients. The cause of rickets/osteomalacia in these patients is a defective 25-hydroxylation due to hepatic impairment and/or decreased vitamin D absorption in these children. Impaired osteoblast function with diminished bone formation and a low serum concentration of 25-hydroxyvitamin D_3 with high PTH levels have been reported in patients with hemochromatosis and liver cirrhosis and in pigs overloaded with parenteral iron.[78-81]

The normocalcemic (25 of 30) patients with β-thalassemia had a slightly higher PTH concentration and significantly lower BMD versus normal children. In agreement with this finding, Pawlotsky et al[82] reported an elevated PTH concentration, normal serum calcium level, and increased bone resorption in their hemochromatic patients. It appears that both vitamin D deficiency and hypoparathyroidism might affect bone mineralization in thalassemic children.

Cytokines

Cytokines represent a group of factors influencing the balance between bone formation and resorption. Increased bone resorption induced by an overproduction of critical cytokines, such as IL-1, TNF, and GM-CSF, by the hyperactive marrow cells and monocyte/macrophage lineage is an attractive theory to explain the pathogenesis of osteoporosis seen in patients with β-thalassemia, as with other diseases. IL-1 and TNF are among the most powerful stimulators of bone resorption known and are well recognized inhibitors of bone formation.[83-87] However, we found normal serum levels of IL-1 and TNF in our thalassemic patients comparable to those for control children, which might rule out a significant role for these cytokines in the development of osteoporosis in these children.

Cortisol Secretion

In this study, children with β-thalassemia had a significantly lower cortisol response to provocation with low-dose ACTH. Other studies reported both low[88,89] and normal[90,91] cortisol responses to high-dose ACTH. Slate gray pigmentation that becomes progressively intense with time, poor weight gain, weakness, and absent adrenarche were significant signs in our thalassemic patients. However, the contribution of different factors including adrenal insufficiency, siderosis, and anemia in the production of these manifestations is difficult to assess. McIntosh[89] reported high circulating ACTH in β-thalassemia, and suggested that it is the cause of the pigmentation. In concert with these findings, the graded-dose adrenal cortical stimulation showed significant suppression of cortisol secretion. Although iron deposition in the adrenals might be the cause of adrenal insufficiency, it has been shown recently that IGF-I enhances the steroidogenesis and ACTH responsiveness of human adrenocortical cells in culture.[92-94] A deficiency of IGF-I synthesis in β-thalassemia might contribute to the defective cortisol production and possibly other adrenal androgens, which might explain the lack of or delay in adrenarche in thalassemic patients. These data suggest that replacement with physiological doses of hydrocortisone might improve some of the manifestations of the disease. In addition, increasing the IGF-I level might also improve the secretion of adrenal androgens necessary for adrenarche.

The question is, what possible therapeutic or preventive options are available that might influence bone mineralization and growth in thalassemic children? The data from this study support the development of a controlled clinical trial to evaluate several possible therapeutic interventions. In addition to proper and aggressive nutritional intervention, which should be an integral part of any treatment strategy, possible new therapeutic interventions would include the following: (1) GH and/or IGF-I replacement therapy, especially for those with GH and/or IGF-I insufficiency. These measures might increase the circulating IGF-I level and consequently increase bone formation and prevent osteopenia. Adding IGFBP3 to IGF-I and/or GH therapy might potentiate their effect on bone growth and mineralization.[67,69-71] (2) Treatment with vitamin D or vitamin D analogs at modest doses (800 to 1,500 IU/d vitamin D_3) may offer a safe and substantial contribution to the prevention of osteoporosis in these children. Positive correlations of vitamin D levels with BMD of the vertebrae and proximal femur have been found in young and old women with poor vitamin D status.[95-98] Patients with osteoporosis and biochemical evidence of rickets need higher doses of vitamin D_3 or its analogs for treatment. (3) Calcium supplementation, which has been shown to increase bone density in normal prepubertal children, is another good potential option.[99] (4) Initiation of puberty at an

appropriate age through the use of progressively increased doses of androgens or estrogens. These agents would prevent osteoporosis and increase the BMD,[23,100-102] forcing into consideration the risk of advancing the bone age faster than the height age.

In summary, prepubertal children with β-thalassemia and normal glucose tolerance have decreased BMD, delayed growth, and a defective GH/IGF-I axis. Biochemical evidence of hypoparathyroidism or rickets may be detected in thalassemic patients with hypocalcemia. It is logical to propose that treatment of these patients with GH and/or IGF-I with aggressive nutritional support and supplementation with vitamin D and/or calcium might improve the bone density and prevent the development of osteoporosis and subsequent fractures.

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Decreased Bone Mineral Density in Prepubertal Children with Sickle Cell Disease: Correlation with Growth Parameters, Degree of Siderosis and Secretion of Growth Factors

by Ashraf T. Soliman,* MD, Haddia Bererhi,** MD, Assim Darwish,* Mahmoud M. Alzalabani,* MD, Yasser Wali,** MD, *and* Behjat Ansari,*** FRCP

**The Department of Pediatrics, University of Alexandria, Alexandria, Egypt*

***The Department of Clinical and Biomedical Physics, SQU Hospital, Muscat, Oman*

****The East Glamorgan General Hospital, Cardiff, Wales, UK*

Summary

Patients with sickle cell disease (SCD) frequently have bone disorders of multifactorial aetiology. We attempted to analyse the relationships between bone mineral density (BMD) on the one hand and auxologic parameters, degree of siderosis, function of the growth hormone (GH)/insulin-like growth factor-I (IGF-I)/IGF-binding protein 3 (IGFBP3) axis, and calcium-phosphate balance in 28 prepubertal children with SCD and 15 age-matched children with constitutional short stature (CSS). Children with SCD had significantly decreased BMD (77.9 ± 11.9 per cent of normal BMD for age and sex) and circulating concentrations of IGF-I (91 ± 31 ng/ml) and IGFBP3 (1.7 ± 0.44 mg/l) compared with the control group (BMD = 93.5 ± 8.2 per cent of normal BMD for age and sex, IGF-I = 221 ± 48 ng/ml, and IGFBP3 = 2.3 ± 0.34 mg/ml). GH response to provocation was defective (peak below 10 μg/l) in 40 per cent of children with SCD. Those with SCD with defective GH secretion had significantly lower circulating IGF-I concentration and BMD than those with normal GH secretion. Serum calcium, phosphate and alkaline phosphatase concentrations were normal in all children with SCD. BMD was correlated significantly with height, weight, and body mass index as well as with the circulating concentrations of IGF-I and IGFBP3. It is suggested that increasing the circulating IGF-I concentration, either through increasing the caloric intake of subjects and/or via GH/IGF-I therapy, may improve growth and bone mineralization in these patients.

Introduction

Peak bone mass is defined as the highest level of bone mass achieved as a result of normal growth. It is one of the principal factors determining bone mass late in life.[1] Studying the normal process and factors influencing bone mass accumulation are essential for understanding childhood growth and development as well as for the prevention of osteoporosis in later life.[2]

Studies have shown that acquisition of bone mineral is gradual in early childhood and accelerates during adolescence until sexual maturity is reached.[3,4] Peak bone mass is mainly achieved by late adolescence, and pubertal development and sex steroids play a crucial role in accumulation of bone mass.[5–7] Recent data indicate that patients with constitutionally delayed puberty had their bone accretion complete by their mid-twenties and their bone mass density does not improve with time.[8,9] Several studies have demonstrated the presence of osteopenia in many chronic childhood conditions.[10–12] Although a variety of factors are of importance, the effect of poor nutrition and delayed puberty appear to be the strongest determinants.[2]

Sickle cell disease (SCD) is associated with many risk factors that can adversely affect bone accretion. These factors include

(1) avascular necrosis during sickling episodes;
(2) delayed growth and pubertal development;[13]
(3) deficiency of important growth factors like IGF-I;[14]
(4) nutritional deficiencies;[15,16] and/or
(5) disturbed calcium metabolism.[17]

Many radiological changes are described in these patients, which become marked with age. These changes combine two features, namely expansion of the medulla

Correspondence: Dr Ashraf T. Soliman, MD, Associate Professor of Pediatrics, University of Alexandria, 3 Abdel Sattar Mansour Street, Loran, Alexandria, Egypt. Fax 00 20 3 587 6828.

of the long bones and the diploic spaces of the cranial vault and the effect of bone infarction and infection. The cortex and trabeculae may be thinned and the inter-trabecular spaces are widened throughout the skeleton. In the small bones of the hands and feet deposition of periosteal new bone along the shaft is combined with irregular areas of translucency within the substance of the bone. The whole bone may be involved. Interference with bone growth can be generalized or localized to the metaphysis of the long bones and vertebral bodies.[18]

Because of the risk factors mentioned above and the significant radiological bone changes in these children, we measured bone mineral density (BMD) in relation to linear growth and secretion of growth factors [growth hormone (GH), insulin-like growth factor-I (IGF-I), and IGF-binding protein-3 (IGFBP3)] in 28 prepubertal children with SCD.

Patients and Methods

Twenty-eight prepubertal children with SCD were the subject of this study. They were randomly selected from the 162 children with SCD attending the Hematology outpatient clinic of the Royal Hospital, Muscat, Oman. All had been on folic acid supplement and vaccinated against pneumococci. None of them had a history of intrauterine growth retardation, any other systemic or endocrine disease, dysmorphic trait, or central nervous system irradiation. Informed consents were obtained from all the parents and when appropriate from the patients before enrolment in the study. Twenty-five age-matched children with CSS served as controls. The protocol of the study was approved by the ethical committee of the hospital. All the children were examined thoroughly with special emphasis on nutritional data. The auxological measurements included weight, mid-arm circumference, and scapular, triceps, and abdominal skin-fold thickness. Harpenden's calipers were used. Height standard deviation scores (Ht SDS) were calculated according to the formula $(x_1 - x_2)/SD$ where x_2 and SD are the mean height and SD respectively, for the matched population, and x_1 is the subject's height. The body mass indices (BMI) were calculated according to the formula weight (kg)/Ht (m^2). The bone age was determined according to Greulich and Pyle's atlas.[19]

After an overnight fast (8 h) venous blood samples were obtained between 8 and 9 a.m. for determination of the complete blood picture, serum albumin, calcium, phosphates, and alkaline phosphate concentrations. The serum was separated from the formed elements by centrifugation and kept frozen at −20°C until analysed for GH, IGF-I, and IGFBP3 by radioimmunoassay. A GH provocation test was performed using oral clonidine (0.15 mg/m^2), and for those who did not respond (peak GH below 10 μg/l), GH stimulation with glucagon 0.1 mg/kg IM was performed. Human GH and IGF-I were measured by radioimmunometric assay, using reagents purchased from Nichols Institute (San Ju Capistrano, CA). Intra-assay coefficients of variati (CVs) averaged 5.6 and 7.2 per cent respectively, a inter-assay CVs averaged 7.8 and 8.5 per ce respectively in the range of GH and IGF-I valu detected. IGFBP3 was measured by radioimmunoass by SCL. Bioscience Services (London) using reager supplied by Mediagnost (Rome). The assay sensitivi was 0.06 μg/ml with intra- and inter-assay CVs of 5 and 8.9 per cent respectively.

Lumbar spine (L-2, L-3, L-4) BMD was measured all patients by dual photon absorptiometry (DPA) usi a Norlad 2600 bone densitometer. All patients we scanned in the supine position. BMD data we expressed in g/cm^2 and were compared with the BM of control and normal children of the same age.[20] Th densitometer was calibrated daily using a phanto standard. The examination was performed with the chi supine, knee flexed, and calves resting on a cushio This positioning separated the spinous processes ar prevented overlap of bony structures. All clothing th could produce artefacts was removed. No sedation restraints were used. The scan took approximatel 15 min. All scans were analysed with the assistance an edge detection program in the scanner software. T minimize variability, one experienced observer onl (H.B.) performed all scan analyses. The coefficient variation on repeated scan analyses was 0.7 per cen The L-2 : L-4 spinal BMD was expressed in g/cm^2.

Data are presented as mean ± SD. Statistical analyse were performed using the unpaired Student's *t*-test fo comparison between the groups. The Wilcoxon test wa used when the data were not normally distributed Correlation coefficients were calculated for each var able against BMD using single and multiple regressio analyses in which BMD was the dependent variable.

Results

Table 1 presents the data for the patients and control According to the peak GH response to provocation, th SCD patients were divided into two groups: group I wit defective GH release (GHD), and group II with norma GH release after provocation. The chronological an bone ages did not differ significantly among the thre study groups. Children with SCD had significantly lowe IGF-I and IGFBP3 concentrations compared to th controls. Those with defective GH secretion had lowe IGF-I and IGFBP3 concentrations compared to thos with a normal GH peak after provocation. Th percentage BMD (measured as a percentage of th normal values for age- and sex-matched children)[20] wa significantly lower in children with SCD compared t controls. The loss of more than 20 per cent of BMI corresponds to a loss of more than 1 SD of bone density The BMD of children with SCD and GHD wa significantly lower than those who had normal GH secretion. BMD was correlated significantly with weigh ($r = 0.547$), height ($r = 0.52$), Ht SDS ($r = 0.369$)

TABLE 1
Growth and laboratory data of patients and controls

	Age (years)	Bone age (years)	BMI (kg/m^2)	Height SD score	IGF-I (ng/ml)	IGFBP3 (mg/l)	Ferritin (ng/ml)	BMD[b] (g/cm^2)	Percentage BMD
SCD with GHD[a] ($n = 12$)	10.1 ± 2.5	8.5 ± 1.1	14.3 ± 1.47	−1.63 ± 0.48	76 ± 22	1.55 ± 0.54	510 ± 140	0.542 ± 0.123	70.1 ± 10
SCD with normal GH secretion ($n = 16$)	8.4 ± 0.8	6.5 ± 1	14.8 ± 1.9	−1.4 ± 0.22	109 ± 23	1.9 ± 0.24	423 ± 86	0.655 ± 0.07	90 ± 6.5
All patients with SCD[c] ($n = 28$)	9.3 ± 1.8	7.7 ± 1.1	14.55 ± 1.7	−1.52* ± 0.4	91* ± 31	1.7* ± 0.44	494 ± 111	0.590 ± 0.09	77.9* ± 11.9
CSS ($n = 25$)	8.7 ± 0.5	6.6 ± 0.9	14.9 ± 0.5	−2.1 ± 0.4	221 ± 48	2.3 ± 0.34	ND	0.675 ± 0.07	93.5 ± 8.2

[a]GHD: peak GH < 10 μg/l in two provocation tests.
[b]BMD: bone mineral density.
[c]SCD: sickle cell disease.
*significant.

BMI ($r = 0.409$), and circulating concentrations of IGF-I ($r = 0.469$) and IGFBP3 ($r = 0.36$) ($p < 0.05$). No significant correlation was found between BMD and serum concentration of calcium ($r = 0.077$), phosphate ($r = 0.027$), alkaline phosphatase ($r = 0.023$), or ferritin ($r = 0.25$, $p = 0.08$).

Discussion

This study demonstrates that children with SCD have significant reduction of BMD. A reduction in BMD of this extent (1 SD below the mean) probably represents a 50–100 per cent increase in the incidence of fractures.[21] We attempted to study some factors that might affect BMD in those patients with SCD, including auxologic data, the degree of siderosis, calcium–phosphate balance, and the GH/IGF-I/IGFBP3 axis.

In this study children with SCD had low BMI (20 out of 28 had BMI below the 10th centile for age and sex) and Ht SD S (18 out of the 28 had Ht SD S ≤2). The significant correlation between BMD on the one hand and weight, height, and BMI on the other suggests that slow growth is an important factor in the production of low BMD in these children. Delayed sexual maturation, a known feature of SCD, can significantly add to the problem by decreasing the peak bone mass.[8,9]

Twelve out of the 28 children with SCD had defective GH secretion after provocation, and as a group ($n = 28$), children with SCD had significantly lower circulating concentrations of IGF-I and IGFBP3 compared to the control group. Children and adults with GHD have reduced BMD and bone formation.[22,23] In those patients, GH replacement increases bone turnover and increases BMD within 6–12 months.[24] Children with SCD and GHD had significantly lower IGF-I and IGFBP3 concentrations and BMD compared with those with normal GH secretion. IGF-I is a GH-dependent polypeptide that circulates in the plasma preferentially bound to the high molecular weight IGFBP3 complex which acts as a reservoir, prolonging the half-life of IGF-I and targeting the IGFs to the relevant target organ. The bioactivity of the circulating IGF-I is therefore very much dependent on the presence of IGFBP3 in the circulation.[25–27] IGF-I is a potent stimulator of bone formation and exogenous administration of IGF-I has been shown to increase bone formation in humans and animals.[28,29] This effect is potentiated when IGF-I is combined with IGFBP3.[30,31] Current opinion favours GH as the major regulator of IGF-I and IGFBP3 levels in humans.[32,33] GH therapy increases both IGF-I and IGFBP3.[33,34] Therefore, GH replacement and/or IGF-I therapy might improve growth and BMD in children with SCD, as seen in other diseases with osteopenia.[30,33]

In addition, serum levels of IGF-I and IGFBP3 are positively related to nutritional status.[35,36] Despite their growth delay, dietary evaluation of our patients with SCD revealed that they have normal qualitative and quantitative food intake compared to age-matched

normal children. This can be explained in part by the increased protein turnover and energy expenditure of these patients, putting them in a hypermetabolic state that requires greater dietary energy compared with normal children (HbAA).[17] One study showed an improvement in both growth parameters and clinical course following caloric supplementation.[15] This nutritional supply exerts some of its effects through increasing IGF-I and therefore might also improve BMD in these patients.[35,36,38] Moreover, in many diseases associated with hypermetabolism there is a state of acquired GH resistance with low IGF-I and IGFBP3 production despite normal or high levels of GH. Preliminary data in animals and humans suggest that GH/IGF-I administration can improve nitrogen economy and anabolism in these conditions.[39]

In this study, the normal serum calcium, phosphate, and alkaline phosphatase concentrations exclude an important role, if any, of disturbed calcium–phosphate balance in the production of low BMD. The degree of siderosis, evidenced by circulating ferritin concentration, was not correlated significantly with BMD. However, the excess iron might inhibit calcium deposition in the bone either directly and/or through decreased hepatic synthesis of IGF-I.[40]

The question is, what possible therapeutic or preventive options are available that may influence bone mineralization in SCD? Obviously proper and aggressive nutritional intervention is necessary to improve growth. Calcium supplements, which have been shown to increase bone density in normal prepubertal children, is a possible option.[41] Growth hormone and/or IGF-I therapy, especially for those with GHD and/or low IGF-I concentration, would seem to be a reasonable option.[33,37] The initiation of puberty at an appropriate age, using oestrogens or androgens, is another option, taking into consideration the risk of advancing the bone age faster than the height age.

In summary, prepubertal children with SCD have decreased BMD, delayed growth, and a defective GH/IGF-I/IGFBP3 axis. It is logical to propose that treatment of these patients with GH and/or IGF-I may improve their linear growth and bone mineralization.

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